THE SPONTANEOUSLY DIABETIC BB RAT: A MODEL OF AUTOIMMUNITY AND IMMUNODEFICIENCY

By

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Dedicated to the memory of

Mr. Mellow

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TABLE OF CONTENTS

Commonly Used Abbreviations

BB	BioBreeding
BB/O	BB rats obtained from Ottawa, Ontario
BB/W	BB rats obtained from Worcester, Mass.
BB x WF	Cross of male BB rat with female WF rat
BB x Lewis	Cross of male BB rat with female Lewis rat
°C	Degrees Centigrade
Con A	Concanavalin A
cpm	Counts per minute
DR	HLA D region-associated antigen
F1	First filial generation
F2	Second filial generation
FCS	Fetal calf serum
HLA	Major histocompatibility complex of man
Ia	Immune response-associated antigen
ICA	Islet cell antibodies (cytoplasmic)
ICSA	Islet cell surface antibodies
IDD	Insulin-dependent diabetes
Ig	Immunoglobulin
IgG	Immunoglobulin Class G
IgM	Immunoglobulin Class M
IL 2	Interleukin 2 or T cell growth factor
MLC	Mixed lymphocyte culture
2-ME	2-mercaptoethanol
MRC OX6	Monoclonal antibody which defines rat Ia-positive cells
MRC OX8	Monoclonal antibody which defines rat cytotoxic/suppressor T cell subset
n.d.	Not done
PBS	Phosphate-buffered saline
PCA	Gastric parietal cell autoantibodies
PHA	Purified phytohemagglutinin
PWM	Pokeweed motigen
RT.1	Major histocompatibility complex of the rat
S.D.	Standard deviation
SMA	Smooth muscle antibodies

WF	Wistar Furth
W3/13	Monoclonal antibody which defines rat T lymphocytes
W3/25	Monoclonal antibody which defines rat helper T cell subset
x	Irradiated

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The BB rat is presently the best available animal model for human insulin-dependent diabetes (IDD). IDD in the BB rat may result from autoimmunity since it is accompanied by lymphocytic inflammation of the pancreatic islets and is preventable by immunosuppression. Antibodies to pancreatic islets (ICA) and other endocrine tissues in BB rats were sought as evidence for an autoimmune etiology for IDD. ICA were not detected, while smooth muscle and gastric parietal cell autoantibodies (PCA) were frequently identified. Although functional abnormalities of gastric parietal cells were not noted, PCA-positive rats had evidence of lymphocytic gastritis. PCA appeared during the age period that

the animals were developing IDD. Thus, the BB rat has an autoimmune diathesis of which IDD may be only one result.

Immunocompetency of the BB rat was also studied. Severe T lymphopenia was observed in all BB rats, irrespective of age or presence of IDD, while numbers of B cells and immunoglobulin levels were normal. Both the numbers of helper T cells and cytotoxic/suppressor T lymphocytes were depressed, and an inversion of the ratio of helper T cells to cytotoxic/suppressor T cells occurred in all BB rats with maturity.

Concomitantly, profound impairments of T cell-mediated immunity were noted to mitogenic stimulation and in allo-responses. BB lymphocytes produced IL 2 normally; however, irradiated WF cells and Con A supernatants did not restore BB responses, suggesting that BB lymphocytes may have defective responses to helper factors such as IL 2. In contrast to BB peripheral T cells, BB and WF thymocytes responded equally well to mitogens. Whereas BB thymic histology was normal, BB spleens and lymph nodes were severely depleted of T lymphocytes. Thymocytotoxic autoantibodies were also detected in many BB rats. These findings suggest that the defect in T cell immunoresponsiveness may be post-thymic or peripherally acquired. Although the BB rat is an intriguing model of autoimmunity and immunodeficiency, no clear relationship between the immunoincompetence and IDD is known to date.

INTRODUCTION

Autoimmune diseases result from loss of self tolerance which leads to an immune response by the individual to autologous antigens and subsequent cellular and tissue destruction or other effects. Such diseases are separable into two groups, i.e. organ specific disorders such as the autoimmune endocrinopathies, and autoimmune diseases that are systemic and not confined to any one organ such as systemic lupus erythematosus.

A disease is generally considered to be an organ-specific autoimmune disorder if there are mononuclear infiltrations of the affected organ or tissue, organ-specific autoantibodies, and a tendency for more than one of these diseases to occur simultaneously in individual patients (1). Such organ-specific autoimmune diseases include chronic lymphocytic thyroiditis, Graves' disease, Addison's disease, acquired hypoparathyroidism and insulin-dependent diabetes (IDD).

Most autoimmune endocrinopathies are associated with disturbed frequencies of certain HLA antigens, especially an increased frequency of the immune response gene HLA DR3 (1,2). HLA DR4 is also increased in IDD (1,2). For some

diseases of this group, such as chronic lymphocytic thyroiditis, however, no definitive HLA associations have been found.

The presence of organ-specific autoantibodies in the patient's serum does not mean the patient has clinical disease nor that the antibodies are actually the cause of tissue damage. However, because the autoantibodies are very specific and are found in low frequencies in the general population, they are considered to be markers or indicators of the presence of an autoimmune disease process occurring in the patient, regardless of whether this process leads to overt disease (3,4).

Proof of a primary role for autoimmunity in the pathogeneses of these putative autoimmune endocrinopathies remains difficult to obtain. Current etiologic concepts for such autoimmune diseases most probably must include discussion of the relationship between environmental triggers and immune responsiveness (as defined by HLA DR antigens) in the genetically predisposed individual.

Insulin-Dependent Diabetes (IDD)

It has been suggested that human IDD may result from the autoimmune destruction of the insulin-secreting beta cells of the pancreatic islets. Evidence to support an autoimmune etiology for IDD has included observations of mononuclear infiltrations in pancreatic islets of patients who have died suddenly after onset of IDD (5,6). These

infiltrative lesions were predominantly composed of lymphocytes, with few polymorphonuclear leukocytes, eosinophils or plasma cells present and are referred to as "insulitis" (7,8). The frequency of insulitis in patients with IDD is variable, with reports ranging from extremely rare (9,10) to greater than 50% (8,11) in patients with recent onset of IDD. Lymphocytic infiltrations of pancreatic islets have not been demonstrated in patients with IDD of greater than one year duration, in noninsulin-dependent diabetics or in normal controls (12).

Islet Cell Autoantibodies in IDD. The presence of antibodies to pancreatic islet cells in sera of patients with IDD and polyendocrinopathies has been extensively described (13,14). Cytoplasmic-reactive islet cell autoantibodies (ICA), detectable by indirect immunofluorescence on normal pancreatic tissue, react with all cell types of the pancreatic islets in addition to beta cells (15-17), which is in contrast to the specific loss of beta cells seen in IDD. ICA do not cross react with gastrointestinal cells secreting hormones also found in pancreatic islets, such as glucagon and somatostatin (2). These antibodies are exclusively of the IgG class, usually fix complement and are believed to react with a microsomal membrane lipoprotein found normally in all islet cells (13,14,18-20). The frequency of ICA in patients with IDD varies according to the patient's race, to the time elapsed after clinical diagnosis of IDD, and has

been found to precede development of the clinical disease (13,15,21,22). Neufeld et al. determined the frequency of ICA to be approximately 74% in Caucasian children tested within three months of onset of IDD, whereas ICA were detected in less than 50% of patients three years after diagnosis (15). However, 10-15% of IDD patients have been noted to have persistent ICA for many years and have been shown to have higher frequencies of associated thyroid, gastric and adrenal autoimmune diseases as well as higher frequencies of HLA B8-bearing haplotypes than patients with IDD who become ICA-negative over similar periods (19,23). In contrast, black insulin-dependent diabetics have only about half the frequency of ICA in relation to duration of IDD as do Caucasian patients with IDD, suggesting that much of the IDD among black patients may be different from IDD seen in Caucasian populations (15,24). In addition, children who are ICA-negative at onset of IDD rarely become positive for these antibodies later (2,3). ICA have also been demonstrated in approximately 3-5% of nondiabetic relatives of IDD probands (15,23) and in about 0.5% of healthy controls (15,23).

Observations that ICA-positive family members of patients with IDD and noninsulin-dependent diabetics with ICA tend to become insulin-requiring with time (22,25,26) suggest that these antibodies may be of clinical value in predicting the subsequent development of IDD (22). However, a causal relationship between ICA and IDD remains to be

proven. A direct role for ICA in the pathogenesis of IDD in fact seems unlikely, especially since these antibodies react with a shared antigen found in the cytoplasm of all islet cell types. Furthermore, autoantibody molecules are not normally considered to be able to cross membranes of living cells to effect damage. In addition, experimental evidence to suggest that ICA do not cause IDD includes findings that transfers of ICA from human patients with IDD to mice have not resulted in IDD in these animals (19,27), nor has placental passage of ICA resulted in the development of IDD in newborn infants or even to affect neonatal insulin secretion (19). Observations that ICA titers decrease with time after clinical onset of IDD is probably related to the progressive loss of the relevant antigen, whatever the disease mechanism.

Islet Cell Surface Autoantibodies in IDD. Autoantibodies reactive to antigens on pancreatic islet cell membranes (ICSA) have also been demonstrated in sera of patients with IDD (21,28-30). Using cell surface immunofluorescence techniques or ^{125}I-labeled protein A assays, ICSA have been detected either by reaction with cultured human insulinoma cells (29), beta cells isolated from dispersed rat or mouse pancreatic islets (21,31,32) or human fetal pancreatic islet cell cultures (33). Analagous to ICA, ICSA have been detected in about 67% of children at onset of IDD and decrease in frequency within the first year after diagnosis (34,35).

In contrast, ICSA are reported to be more common than ICA in controls and seem to possibly occur independently of ICA in patients with IDD (36).

ICSA in sera from patients with IDD have been shown to be cytotoxic to mouse pancreatic islets in vitro (37), however some nondiabetic sera without ICSA were also cytotoxic to these cells. Sera from IDD patients have also been shown to have complement-mediated cytotoxic effects on hamster islets (38) and rat islets (39), correlating with the detection of ICSA in the serum. In addition, ICSA-positive sera in the presence of complement have been demonstrated to cause increased chromium release from labeled rat islet cells (40,41) while sera containing ICA alone did not have such effects (40). Difficulties with this study include defects in the method of ICA detection, and findings that 25% of ICSA-positive sera from nondiabetic first degree relatives were also cytotoxic to islet cells.

There has been little convincing evidence to suggest that ICSA react specifically with pancreatic beta cells. Indeed in one study, cytotoxicity of sera with detectable ICSA was shown to not be restricted to pancreatic beta cells, but also affected a somatostatin-producing tumor line (42). However, Dobersen and Scharff has recently demonstrated the preferential lysis of rat beta cells with minimal killing of other types of islet cells by ICSA-containing diabetic sera using double-label immunofluorescence techniques (28). In addition, eleven of twenty-one

sera from diabetic patients with ICSA were shown to be able to suppress glucose and theophylline-stimulated insulin release but not glucagon release in vitro by dispersed mouse islets (43). Paradoxically, ICSA have been demonstrated to have a stimulatory effect on basal insulin release from cultured mouse islets (44). These results are consistent with the primary role for ICSA in beta cell destruction seen in IDD proposed by some investigators (28), but much more substantial evidence is needed.

Cell-Mediated Autoimmunity in IDD. No consistent generalized defects in cell-mediated immunity as defined in terms of lymphocyte responsiveness to phytohemagglutinin (PHA) (45-48) and in the enumeration of lymphocyte subpopulations (45,49-51) appear to be present in well-treated patients with IDD. However, poorly controlled diabetics have been observed to have depressed mitogenic responses to PHA in comparison with matched adequately treated IDD patients and healthy controls (47,52) suggesting that the metabolic derangements of IDD have an adverse effect on T lymphocyte responsiveness. Other studies have indicated that specific antipancreatic cell-mediated immunity may be observed in patients with IDD (45,47). Nerup et al. demonstrated significant inhibition of migration of leukocytes from insulin-dependent diabetics in the presence of porcine pancreas (53) or fetal calf pancreas (54,55) homogenates. Abnormal migration inhibition was especially noted in those

patients with IDD for less than one year, but was also demonstrated in some noninsulin-dependent diabetics. Positive leukocyte migration inhibition in IDD patients has also been observed using human pancreas homogenates (56) or insulinoma extracts (57) as antigens. Concomittantly, delayed hypersensitivity skin reactions to porcine pancreatic suspensions were seen by Nerup in patients with IDD who exhibited inhibition of leukocyte migration using the same antigen (53). In order to examine the possible role of cell-mediated immunity to insulin and thus to beta cells in the pathogenesis of IDD, MacCuish (47) and others (58) were able to demonstrate significantly greater blastogenesis, as measured by ^{3}H-thymidine incorporation, by lymphocytes from diabetic patients when cultured with bovine or porcine insulin than by lymphocytes from control patients.

Huang and Maclaren were able to demonstrate specifically enhanced cytoadherence and cytotoxicity of human insulinoma cells in vitro by peripheral blood lymphocytes from children with IDD (59). Specific insulinoma-tumor cell cytotoxicity, as measured by eosin exclusion, was seen both with and without added patient sera, but was not obtained using either lymphocytes from patients with systemic lupus erythematosus or autoimmune thyroid disease, or with different tumor lines as targets (59,60). Peripheral blood lymphocytes from twenty-one out of thirty-three patients with IDD were shown to inhibit insulin release by rat islets to glucose and theophyllin (61), while no inhibition of insulin

secretion was noted by lymphocytes from noninsulin-dependent diabetics or controls. Significantly increased levels of circulating killer (K) cells, classified as low affinity E-rosette forming cells, were also found in 57% of newly diagnosed insulin-dependent diabetics (62). These levels returned to normal within twelve months from diagnosis of IDD. Raised K cell numbers were accompanied by significantly enhanced levels of antibody-dependent cell-mediated cytotoxicity activity to chromium-labeled human erythrocytes sensitized with antierythrocyte antibodies in many IDD patients (63).

Several investigators have suggested that defects in suppressor T cell activity were present in patients with IDD (64,65). In one study, Concanavalin A (Con A)-activated lymphocytes from patients with IDD poorly suppressed allogeneic mixed lymphocyte cultures when compared to suppressor activity demonstrated by Con A activated lymphocytes from controls (64). In contrast, Slater et al. recently found a statistically significant increase in Con A activated suppressor T cell activity in thirteen patients with IDD (66). These measurements of cell-mediated immunity, however, are difficult to analyze even in healthy people, and biologically significant results with diabetic patients are even more questionable because the effects and complications of IDD itself affect lymphocyte function and responsiveness.

In a provocative study, indium-labelled autologous peripheral blood leukocytes from two of three newly diagnosed patients wtih IDD were observed by CAT scanning to become distributed in the same patient's pancreas after intravenous reinjection (67). No pancreatic localization using the same procedure was noted in scans of patients with other diseases. Whether the lymphocytes specifically homed to the pancreas or were trapped there nonspecifically as a consequence of inflammation, these results suggest the presence of pancreatic insulitis in these patients.

Associated Autoimmune Diseases. Other organ-specific autoimmunities are found with increased frequencies in IDD (1,19,68,69). These diseases mainly involve the thyroid, the adrenal and the parietal cells of the gastric mucosa. In studies by Riley and colleagues of more than 500 children with IDD, 17% of the patients had detectable thyroid microsomal antibodies and 5% had overt thyroid disease in comparison with less than 2% of the control population even having thyroid antibodies (15). Adrenal autoantibodies and Addison's disease were also more frequent in Caucasian children with IDD, being found in 2% and 0.5% of insulin-dependent diabetics respectively, compared to adrenal antibodies being detected in 0.7% of matched controls (15,69). Finally, gastric parietal cell autoantibodies were detected in approximately 9% of patients with IDD and in only 1% of matched controls (19). Children with other autoimmune

endocrinopathies in addition to IDD have even higher frequencies of associated organ-specific autoantibodies (19). For example, patients with both IDD and thyroid microsomal antibodies have augmented incidences of adrenal antibodies to about 6% (19). Organ-specific autoantibodies and autoimmune disease are not found in increased frequencies in noninsulin-dependent diabetics (19).

HLA Associations with IDD. Singal and Blajchman first reported that IDD was associated with disturbed frequencies of HLA antigens and noted an increase in HLA B15 in these patients (70). Nerup and coworkers later documented statistically significant increases in HLA B8 and HLA B15 in patients with IDD in comparison with matched controls, while no HLA differences were seen between controls and noninsulin-dependent diabetics (71). Subsequent studies have both confirmed and extended these observations (72-74). Cudworth (75) and others (76) have postulated that there are two HLA haplotypes associated with increased relative risks for IDD: HLA A1 (A30) B8 (B18) Cw3 DR3 and HLA A2 B15 (B40) DR4. The primary association of IDD seems to be with the HLA DR antigens and secondarily due to linkage disequilibrium with the HLA A and B antigens (2,72,73,77). HLA DR3 is found in 36-59% of IDD patients compared to between 11-24% in normal controls (72), while HLA DR4 is seen in 32-58% of insulin-dependent diabetics compared to 16-28% in the general population (72). Patients with multiple autoimmune

endocrinopathies have an even higher frequency of HLA A1 B8 DR3 haplotypes and especially HLA DR3, suggesting that a gene predisposing for general organ-specific autoimmunity is associated with HLA DR3 (2,45). The risk of developing IDD is highest for HLA DR3/DR4 heterozygotes, implying the possibility of the existence of at least two hereditary susceptibility genes for IDD associated wtih HLA genes--one associated with HLA DR3 and the other with HLA DR4 (34,45, 77,78). One haplotype has been suggested to possibly render protection against IDD because it is decreased and virtually absent in Caucasian patients with IDD: HLA A3 (A11) B7 DR2 (2,75,77,78), although the low frequencies of HLA DR2-bearing haplotypes in IDD may be due in part to the increased frequencies of HLA DR3 and HLA DR4 (79). Black patients with IDD as a whole do not have as significantly disturbed frequencies of HLA DR3 and DR4 antigens as Caucasian children with IDD (24). However, those black diabetic children who are positive for ICA seem to invariably type HLA DR3 or HLA DR4 (24), which is probably due to the impact of Caucasian IDD genes in the black genome by racial admixture (24). Families with multiple members affected by IDD tend to show an excess of individuals typing for HLA DR3 or HLA DR4. In addition, several investigators (80-82) have observed significantly disturbed frequencies of several complement factor B alleles in insulin-dependent diabetics (2). The mechanisms through which HLA antigens, especially the immune response associated HLA DR antigens, affect susceptibility to IDD remain to be elucidated.

Mechanism of Inheritance of IDD. The apparent linkage between IDD and HLA has made it possible to analyze the segregation of HLA haplotypes as markers for IDD in multiplex families with two or more siblings with IDD. Several investigators have suggested IDD to be a recessive disease (83,84), while others believe IDD to be dominant. In either case, reduced penetrance would need to be invoked to explain the segregation of IDD with HLA haplotypes (85,86). Indeed, the penetrance or the percentage of people carrying the IDD susceptibility gene(s) that actually have clinical IDD seems to be quite low, approximating 15-30% in multiplex families (86). Speilman has recently suggested a hypothesis of differential susceptibility to IDD depending on dosage of IDD susceptibility gene(s), rather than simple dominant or recessive inheritance (87). Heterozygosity of IDD alleles would result in significant susceptibility to IDD, but homozygosity for the gene(s) would be associated with even greater risk (penetrance) for the disease. However, because of uncertainty as to the random frequency of IDD genes in the general population, the crossover rates between HLA and IDD gene loci (if not one and the same), and the probable genetic heterogeneity of the disease, estimations of the mode of transmission of IDD are difficult to make (19).

Environmental Factors in the Pathogenesis of IDD. As the concordance for IDD in monozygotic twins has been shown by Leslie and Pyke to be at most 50% (88), environmental

influences have been implicated as at least secondary factors in the development of IDD (86). Support from animal models for a viral role in the pathogenesis of IDD includes evidence that encephalomyocarditis virus causes a diabetes-like syndrome in susceptible SJL mice, associated with beta cell loss and pancreatic insulitis (89).

There is limited direct evidence for such viral participation in human IDD. Coxsackie B4 virus was isolated from the pancreas of a child with fatal diabetic ketoacidosis which proved capable of inducing insulitis and hyperglycemia in some strains of mice (90). An epidemiological relationship between annual cycles of infection with Coxsackie B4 virus in people and the seasonal incidence of IDD has also been found (91), and a high frequency of IDD in children who have suffered severe congenital rubella or mumps infection has been reported (45,92). One study has suggested that the frequency of IDD in patients after infections with these viruses is positively associated with HLA B8 and thus by linkage disequilibrium with HLA DR3 (93). If confirmed, such findings would suggest a relationship between the immune response of an individual to a virus and the development of IDD. Several investigators suggest that these viruses do not usually cause IDD, but instead may trigger IDD by a stress effect in the susceptible prediabetic individual with preexisting insulinopenia. To this effect, it is notable that no epidemics of IDD have been reported in several large registry studies in the United States, London and Denmark.

The BB Rat

The spontaneously diabetic BB rat was first recognized in 1974 in an outbred colony of Wistar rats at the BioBreeding Laboratories (94). These rats have subsequently been bred for the IDD phenotype. BB animals which have been formally inbred for seven to twelve generations develop spontaneous severe IDD at about 70-120 days of age, which is characterized by insulinopenia, marked hyperglycemia, ketoacidosis, weight loss and an absolute requirement for exogeneous insulin (95). The BB rat most closely resembles human IDD of all animal models known to date. IDD develops in genetically susceptible male and female rats with equal frequencies (95,96) and is thought to be inherited either as a single autosomal recessive gene with reduced penetrance (94) or as multiple genes.

Moderate insulitis is seen in the pancreatic islets of BB rats at the time of diagnosis of IDD, resembling the pancreatic lesions seen in recently diagnosed human patients with IDD (96,97). Insulitis has also been observed in nondiabetic animals (94). Immunochemical staining of pancreatic islets from diabetic BB rats for insulin content reveals depletions of beta cells, especially marked in pancreases from BB rats with longstanding IDD (98,99). Such pancreatic islets are comprised almost exclusively of glucagon, somatostatin and pancreatic polypeptide-secreting cells (94).

The IDD seen in the BB rats is probably not due to recognized infectious agents, since animals raised in a gnotobiotic environment, such that they did not develop antibodies to any bacteria, viruses or parasites, did not have decreased incidences of IDD (100). However, this study was based on results from only one litter and, in any event, does not rule out the possibility of a role for a vertically transmitted virus in the etiology of IDD in these rats.

The typical insulitis lesions and the genetic predisposition for IDD suggest that IDD in the BB rat may be the result of beta cell autoimmunity. Evidence to support such a hypothesis includes recent findings of ICSA in twelve of fourteen diabetic BB rats using a ^{125}-labelled protein A assay (101). Other data include observations of the reduction in frequency of IDD in susceptible BB rats after administration of antilymphocyte serum (102), neonatal thymectomy (103,104) or bone marrow reconstitution (105). However, the experimental designs were questionable since the studies were performed between groups of litters rather than within litters. Because the incidence of IDD varies from 0-60% in any one litter to the next, the observed decrease in frequency of IDD, but not total IDD prevention, may instead be due to litter assignment rather than treatment. In addition, no research group has yet been able to successfully transfer IDD immunologically from BB rats to nondiabetic recipients.

Less direct evidence for an involvement of autoimmunity in the pathogenesis of IDD in the BB rat includes recent findings by Colle and coworkers of a genetic linkage between IDD and the rat major histocompatibility complex RT.1 in F2 animals produced by initial matings of male BB rats with IDD and RT.1 incompatible female Lewis rats (106). BB rats have also been shown to have decreased numbers of circulating T lymphocytes (107) and to be extremely susceptible to opportunistic infections. As is the case with human IDD, more evidence is needed in order to prove an autoimmune etiology for the loss of pancreatic beta cells and thus IDD in the BB rat.

SPECIFIC AIMS

From the previously mentioned findings, IDD in the BB rat and in humans is thought to result from pancreatic beta cell autoimmunity. However, much more evidence is required to substantiate this hypothesis. In order to better understand the etiology and genetics of IDD in the BB rat and its similarities to human IDD, the following studies were performed.

1. Identification of organ-specific autoantibodies in BB rat sera. Several of such antibodies occur with increased frequencies in human IDD and their presence in BB rats would support a role for autoimmunity in the strain.
   a. Autoantibodies to the cells of the pancreatic islets, the thyroid, the adrenal gland, and the gastric mucosa were sought in BB rats from an early age before the development of IDD in order to determine whether a correlation between IDD onset and the appearance of autoantibodies existed.
   b. Clinical evidence of disease was determined in those rats with organ-specific autoantibodies.

c. Crosses between BB and WF rats were performed in order to indicate the inheritance of autoantibodies and their relationship to IDD.

2. Evaluation of the immune system of the BB rat. These studies were done in the hopes of identifying abnormalities which could both predispose to autoimmune disease and explain the increased susceptibility of these animals to infection. Both diabetic and nondiabetic BB rats were studied.

   a. The various circulating leukocyte populations and major lymphocyte subsets were counted in BB rats and compared to numbers observed in WF and BB x WF F1 animals.

   b. The ability of the immune system of BB rats to function in vivo was determined by observing how well these rats could reject skin grafts involving both major and minor histocompatibility differences.

   c. The ability of BB lymphocytes to function in vitro was tested by the responses of lymphocytes to mitogens or to allogeneic cells in mixed leukocyte cultures (MLCs).

   d. The possible presence of increased suppressor activity in BB rats and the ability of BB lymphocytes to produce and respond to helper factors such as interleukin 2 (IL 2) were determined.

e. The proliferative responses of BB and WF thymocytes to mitogens were compared.

f. Autoantibodies to thymocytes were sought in sera from BB rats.

g. Histological examination of thymuses, spleens, and lymph nodes from BB rats were made.

h. Gamma globulin levels were measured in BB rats as a gross indication of B lymphocyte function.

3. Attempts to transfer IDD with pancreas extracts and cells from spleens and lymph nodes from BB rats to nondiabetic BB, WF and BB x WF F1 rats were performed. Positive findings would thus provide convincing proof of an autoimmune etiology for IDD in the BB rat.

4. Evaluation of the genetics of IDD and autoantibodies in the BB rat.

   a. Different mating combinations were performed between BB rats with and without IDD and/or autoantibodies in order to observe the modes of inheritance of these autoimmune parameters.

   b. Crosses between male BB rats with IDD and both female WF and Lewis rats were performed in order to observe the relationships between IDD, autoantibodies, and the rat major histocompatibility complex, RT.1.

MATERIALS AND METHODS

Animals. One hundred and fifty BB rats of both sexes and of varying ages in addition to Wistar Furth (WF) rats (Charles River Laboratories, Wilmington, MA) and Lewis rats (Charles River) were used in these studies. Initially, 40 BB rats from 15 litters were obtained from Dr. Pierre Thibert (Animal Resources Division, Health Protection Branch, Ottawa, Ontario) and 28 BB rats from 3 litters were obtained from Dr. Arthur Like (University of Massachusetts, Worcester, MA). The animals obtained from Dr. Thibert and Dr. Like are referred to as BB/O and BB/W rats respectively. The remaining 82 BB rats were bred in our laboratory from the original animals obtained above. Male BB rats with IDD were also mated with inbred WF or Lewis females. Male and female F1 progeny were then interbred to produce F2 rats.

The animals were given Purina Rodent Chow 5001 and water ad libitum, with light regulation at 0730 and 1830 hours. Diabetic rats were maintained on PZI insulin given between 1500 and 1700 hours daily by subcutaneous injections into axillary skin folds at doses sufficient to sustain weights, minimize polyuria and avoid clinical hypoglycemic episodes.

Detection of IDD and Sera Collection. All BB rats had blood and urinary glucose levels determined weekly. Blood samples were drawn from the periorbital venous sinus using heparinized capillary tubes while the rats were under light ether anesthesia. After clotting, serum glucose levels were run in duplicate on a Beckman II glucose analyzer (Beckman Instruments, Inc., Fullerton, California). Sera were collected weekly and then stored at -20°C until testings for autoantibodies were made. Rats were considered to have IDD if the animals had glycosuria or serum glucose levels above 250 mg/dl. For some studies, intraperitoneal glucose tolerance tests (1.75 g/kg) were performed after an overnight fast on BB rats aged 6-9 months who had not developed overt IDD.

Handling and Preservation of Tissues for Autoantibody Detection.

Normal WF rat tissues were used for detection of organ-specific autoantibodies. After removal, the tissues (thyroid, pancreas, adrenal and stomach) were cubed and immediately snap frozen in isopentane cooled in a mixture of dry ice and acetone and then stored at -80°C. Four micrometer tissue sections were cut out on a SLEE HR Mark II cryostat (Slee Medical Equipment Ltd., London, England) at -20°C, placed on slides and air-dried. The slides were either used immediately or stored for no more than one month at -80°C.

Tissue Histology. Thymuses, spleens, lymph nodes, stomachs and pancreases were removed from BB, WF and BB x WF F1 rats and placed in a 10% formalin solution. The final paraffin-embedded tissue sections were stained with hematoxylin and eosin before histological examination.

Isolation of Peripheral Blood Mononuclear Cells. One to 3 ml blood samples were withdrawn from the periorbital venous sinus or tail vein into 3 ml EDTA vacutainer tubes (Becton-Dickinson, Rutherford, NJ) while the rats were under ether anesthesia. The blood was diluted with phosphate-buffered saline (PBS), layered over a Ficoll-Hypaque gradient (24:10 v/v; 9% Ficoll 400, Pharmacia, Piscataway, NJ: 34% Hypaque M, Winthrop Laboratories, New York, NY), and the mononuclear cells (PBL) isolated at the interface after centrifugation at 2200 rpm for 13-15 minutes. Any remaining erythrocytes were lysed by exposure to an ammonium chloride-Tris buffer solution for 3 minutes at 37°C. The PBL were then washed twice in PBS before adjustment to the desired cell concentrations in either PBS for T cell subset determinations or in complete medium: RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 50 μg/ml gentamycin (Schering, Kenilworth, NJ), 5% heat-inactivated fetal calf serum (FCS) (GIBCO), and 5×10^{-5}M 2-mercaptoethanol (2-ME) (Bio-Rad, Richmond, CA) for mitogen and MLC assays. Viability of cells was measured by trypan blue exclusion.

Isolation of Splenic Lymphocytes. The spleens were removed asceptically from anesthesized rats, minced, pressed through nylon mesh screens and resuspended in cold PBS. Any cell clumps were dispersed by repeatedly aspirating the suspensions through a series of varying gauge (19,21,23,25) needles, after which the cell suspensions were centrifuged at 1600 rpm for 10 minutes at 20°C. Erythrocytes were removed as stated previously. After washing the pellets twice in PBS, the cells were resuspended in either PBS for transfer experiments or in complete medium for in vitro assays.

Preparation of Purified Splenic Lymphocytes. For some experiments, spleen cells were purified of F_c receptor-bearing T cells and Ig-positive B cells and monocytes by passage through rabbit immunoglobulin (Ig)-antirat Ig-coated glass bead columns, prepared according to the protocol of Wigzell et al. (108). After passage through the columns, the remaining cells were washed in PBS and resuspended in complete medium. The purified spleen cell populations were observed to be 98% W3/13 monoclonal antibody reactive cells with less than 2% MRC OX8-positive cells and B lymphocytes present. (See below under leukocyte populations and T cell subsets for discussion of monoclonal antibodies).

Preparation of Thymocytes. Thymocyte suspensions were prepared using the above method for splenic lymphocytes, but

were resuspended in RPMI 1640 without FCS or 2-ME when used in microcytotoxicity assays.

Preparation of Pancreas and Peritoneal Cavity Suspensions. Pancreases were removed aseptically, rinsed in PBS, minced and pressed through nylon mesh screens. The suspension was then centrifuged at 1600 rpm for 10 minutes at 20°C and resuspended in 1 ml of PBS. Peritoneal cells were obtained by rinsing the open cavity with PBS and withdrawing the fluid by pipette. This procedure was repeated several times until the fluid removed from the peritoneal cavity was clear. The suspension was then spun and resuspended in 1 ml of PBS.

Production of Con A Supernatants. Spleen cells from both BB and WF rats at concentrations of 4-5 x 10^6 cells/ml were incubated with 5 μg/ml Con A in complete medium for 16-20 hours at 37°C in 25 cm^2 tissue culture flasks (Corning, Medfield, MA). The contents of the flasks were then centrifuged at 2000 rpm for 10 minutes, the cell-free supernatants (Con A sups) removed and filtered through 0.45 μm filters (Sybron/Nalge, Rochester, NY) and stored at -70° C for future use.

Detection of Organ-Specific Autoantibodies. BB and WF rat sera were applied undiluted to slides of air-dried, unfixed pancreatic or adrenal tissue and at 1:4 dilution and

at 1:10 dilution to thyroid and stomach sections respectively. Optimal results (lowest background and highest sensitivity) were obtained when these specified dilutions were used on the various tissues. After incubation with rat sera for 30 minutes in the dark, the slides were washed three times in PBS. Rabbit antirat IgG-fluorescein isothiocyanate conjugate (Cappel Laboratories, Cochranville, PA) was then added at 1:60 final dilution to the slides and incubated for 60 minutes in the dark. After washing in PBS, the slides were dried, covered with glycerol and glass slips and read under a Leitz Dialux 20 ultraviolet glass microscope fitted with an HB-100 mercury lamp and KP490, TK510 and K515 filters (E. Leitz, Inc., Rockleigh, NJ). All results were read double blind with control negative and antibody-positive sera in each batch. Sere were considered to have autoantibodies if positive immunofluoresence of the tissue was observed. Sera were tested for the presence of autoantibodies at least monthly after the rats were 40 days of age.

Measurement of Gastric Acidity and Serum Iron and Vitamin B12 Levels.

Serum vitamin B12 levels were measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Serum iron levels and iron binding capacities were measured by the ACA-III autoanalyzer (Dupont Inc., Wilmington, DL). Gastric acidity after pentagastrin administration (0.06 mg/kg body weight)

was determined by gastric aspirates during the fasting state and was quantitated by color changes of pH indicator paper.

Transfer Studies. Spleen, mesenteric lymph nodes and peritoneal suspensions from BB rats 1 to 10 days after onset of IDD were injected into the tail veins of anesthesized recipient nondiabetic BB, WF or BB x WF F1 rats using 25 gauge butterflys (Deseret, Sandy, UT). Pancreas suspensions were given intraperitoneally. In some experiments, the recipients were given 300-350 rads of irradiation 24 hours before the transfer. Blood glucose levels were measured on sera from recipient rats on day 0, day 3 and every week thereafter for at least 90 days. Autoantibodies to the cells of the pancreatic islets, the thyroid gland, and the gastric mucosa were also sought monthly in sera recipient rats. After approximately 90-120 days, the recipients were sacrificed and the pancreases removed for histological examination.

Determination of Leukocyte Populations and Rat T Cell Subsets.

White blood cell counts were determined on whole blood after 1:200 dilution in acetic acid using a hemacytometer, and differentials were made from slides of Wright-Giemsa stained cells.

Monoclonal antisera to the various T cell subpopulations were kindly provided by Dr. Alan Williams (Medical

Research Council Cellular Immunology Unit, Oxford, England). The specificities of the antibodies were known to be as follows: W3/13 - all T lymphocytes, W3/25 - helper T cells, MRC OX8 - cytotoxic/suppressor T cells, and MRC OX6 - Ia-positive cells (109). Aliquots of 1-2 x 10^6 PBL were incubated at 4°C for 30 minutes with 1:20 dilutions of these antisera in addition to a 1:15 dilution of rabbit antirat Ig (Accurate Chemicals, Westbury, NY) for determination of the numbers of B cells and monocytes, and PBS or normal rat serum alone as a control. After 2 washings with cold PBS, all PBL aliquots were incubated with a 1:20 dilution of a fluorescein isothiocynate - conjugated goat antimouse IgG (Cappel Laboratories) for 30 minutes at 4°C, washed in cold PBS, and resuspended in 30 µls of PBS-glycerol. Slides were then prepared and were observed for positive immunofluorescence under the ultraviolet microscope.

Detection of Thymocytotoxic Autoantibodies. Two µl samples of sera from both diabetic and nondiabetic BB animals of varying ages, WF rats and Lewis rats were applied undiluted or at 1:2 dilution to 72 well microcytotoxicity trays (Falcon, Oxnard, CA). A modification of the microcytotoxicity method of Amos was used for determination of thymocytotoxic autoantibodies (110). One µl samples of either a BB or WF thymocyte suspension at a concentration of 2 x 10^6 cells/ml were added to each well and incubated with the various sera for 30 minutes at room temperature.

The cells were then washed in PBS and incubated with 4 µls of a 1:10 dilution of guinea pig serum (Dutchland Laboratories, Denver, PA) for 60 minutes at room temperature. After washing and staining with a 1% trypan blue solution, the plates were read for determination of positive cytotoxicity (greater than 50% cell death in any well).

Skin Graftings. Sections of ear pinnae were removed from anesthesized nondiabetic BB, WF or Lewis rats, split in half, and washed in sterile PBS. Sections of skin conforming to the shapes of the ear grafts were removed from the sides of shaved, anesthesized BB or WF rats. After washing the wounds with sterile PBS, the ear grafts were placed onto the prepared areas with the hairless sides down and lightly sprayed with a plastic dressing (Aeroplast, Parke-Davis, Greenwood, SC). Gauze bandages were wrapped around the rats and left in place for one week and then removed. The grafts were considered to have taken if no macroscopic evidence of necrosis was apparent at this time. Skin grafted rats were then followed for evidence of rejection by daily inspection.

Mitogen Assays. In most mitogen or MLC experiments, only nondiabetic BB rats were used due to the possible effects of hyperglycemia on lymphocyte responsiveness. However, a few experiments were performed using well controlled diabetic BB rats in order to see if similar results would be obtained. Unseparated or purified (rabbit Ig antirat Ig treated)

splenic lymphocytes or PBL from nondiabetic or well controlled diabetic BB and WF rats at various cell concentrations ranging from 0.3-2 x 10^5 cells/well were cultured in round-bottom microtiter plates (Costar, Cambridge, MA) with several mitogen concentrations. Pokeweed mitogen (PWM; 1-25 μg/ml) (Sigma, St. Louis, MO), PHA (0.025-1%) (Difco, Detroit, MI), Con A (0.5-10 μg/ml) (Miles-Yeda, Rehovoth, Israel) and WF Con A sup (0.1/ml/well) were used for a total volume of 0.2 ml of complete medium in each well. After 48 hours incubation at 37°C in 5% CO_2, the cultures were pulsed with 1.0 μCi/well of ^{3}H-thymidine (Schwarz/Mann, Spring Valley, NY, specific activity of 6 Ci/mM) and harvested 18 hours later onto filter paper with a 24-line cell harvester (Otto Hiller, Madison, WI). The filters were then air-dried, placed into vials containing scintillation fluid, and counted in a LKB Model 8100 liquid scintillation counter (LKB Instruments, Rockville, MD). In several experiments, 0.5 x 10^5 spleen cells irradiated with 3000 rads from a ^{137}Cs source (Gammator Model M) were also added to some wells. Some 0.5 x 10^5 or 1 x 10^5 BB and WF thymocytes/well were also incubated with 0.1 μg/ml and 1 μg/ml Con A and 0.1 ml/well WF Con A sup for 48 hours at 37° C, pulsed with 1.0 μCi/well of ^{3}H-thymidine and harvested 18 hours later.

Mixed Leukocyte Cultures (MLCs). MLCs were performed in round-bottom microtiter plates, with each well containing

$0.25\text{-}1 \times 10^5$ unseparated or purified responder spleen cells and $0.5\text{-}3 \times 10^5$ irradiated (3000 rads) unseparated spleen cells as stimulators. In addition, third-party irradiated or nonirradiated unseparated spleen cells at a concentration of either 0.25×10^5 or 0.5×10^5 cells were added to some wells with a final volume in each well always of 0.2 ml of complete medium. The plates were incubatd at 37°C in 5% CO_2 for 5 days, pulsed with 1.0 µCi/well of ^{3}H-thymidine as previously described, and harvested 18 hours later. Also, 0.1 ml of WF Con A sup was also added in some cases to wells containing either responders alone or both responder and stimulator cells.

Interleukin 2 (IL 2) Assay. Levels of IL 2 in Con A sups from both BB and WF rats were determined by the stimulatory activity of these samples on a murine IL 2-dependent cytotoxic T cell line (anti-EL4, generously provided by Dr. Shiro Shimuzu, University of Florida). Some 20×10^3 cytotoxic T cells in 0.1 ml of complete medium were incubated at 37°C for 22 hours with 0.1 ml of various Con A sups and 6 dilutions (50%-1.5%) of a reference murine Con A sup. The cultures were then pulsed with 0.5 µCi/well of ^{3}H-thymidine and harvested 6 hours later.

Measurement of Gamma Globulin Levels. Gamma globulin levels were determined on 10 µl serum samples from BB rats with and without IDD, and WF rats using the Beckman microzone serum protein electrophoresis system.

RESULTS

Age at Onset and Frequency of IDD. The frequency of IDD was determined in the original 20 BB/O and 28 BB/W rats. The incidence of IDD was found to be 80% (16/20) in BB/O rats, while IDD developed in 75% (21/28) of BB/W animals (Table 1). However, two female BB/O rats greater than 285 days of age and one male BB/W rat who had reached 180 days of age without developing overt IDD, had one hour peak glucose levels of 513 mg/dl, 671 mg/dl and 298 mg/dl respectively, after glucose loading. Peak glucose levels seen in three control rats did not exceed 220 mg/dl after glucose loading (data not shown). Thus, these three BB rats were considered to have noninsulin-dependent diabetes.

No significant sex difference was seen in the frequency of IDD in either BB/O or BB/W animals. The time period for onset of IDD in the BB/O rats was from 90 to 120 days of age (Figure 1), while the BB/W rats had a broader age range for onset of IDD of between 70 and 161 days (Figure 2). All BB rats were studied beyond 180 days of age, which exceeded the critical age span for development of the disease in these rats. Mild to moderate insulitis was seen in pancreases from BB rats at onset of IDD (Figure 3). None of the

TABLE 1

IDD AND AUTOANTIBODIES IN BB/O, BB/W, WF AND BB x WF F1 HYBRID RATS

Rats	IDD %	Autoantibodies ICA %	PCA %	SMA %	TCA %[b]
BB/O (n=20)	80	0	35	55	5
BB/W (n=28)	75	0	68	61	18
BB x WF F1 (n=50)	0	0	0	21	0
WF (n=30)	0	0	0[a]	7	0

[a]One rat had equivocal PCA. All rats ascertained for the above were studied up to and beyond 6 months of age.

[b]Thyroid colloid autoantibodies.

FIGURE 1. Frequencies of insulin-dependent diabetes (IDD), gastric parietal cell antibodies (PCA) and smooth muscle antibodies (SMA) in 20 BB/O rats with age.

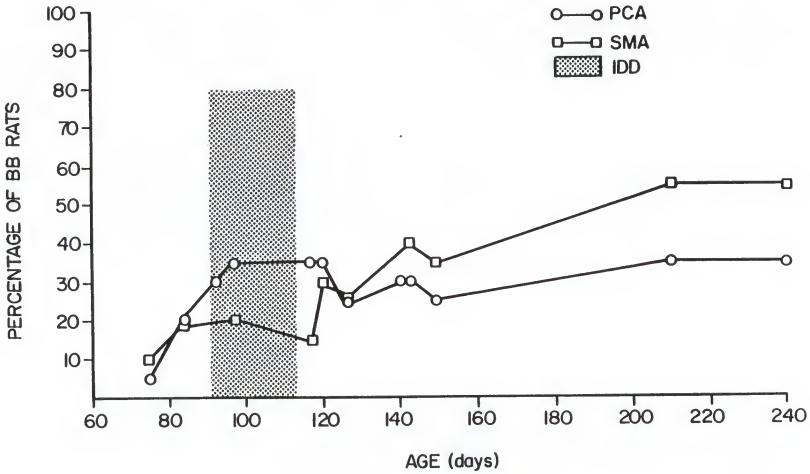

PCA
SMA
IDD
PERCENTAGE OF BB RATS
100
90
80
70
60
50
40
30
20
10
60
80
100
120
140
160
180
200
220
240
AGE (days)

FIGURE 2. Frequencies of IDD, PCA and SMA in 28 BB/W rats with age.

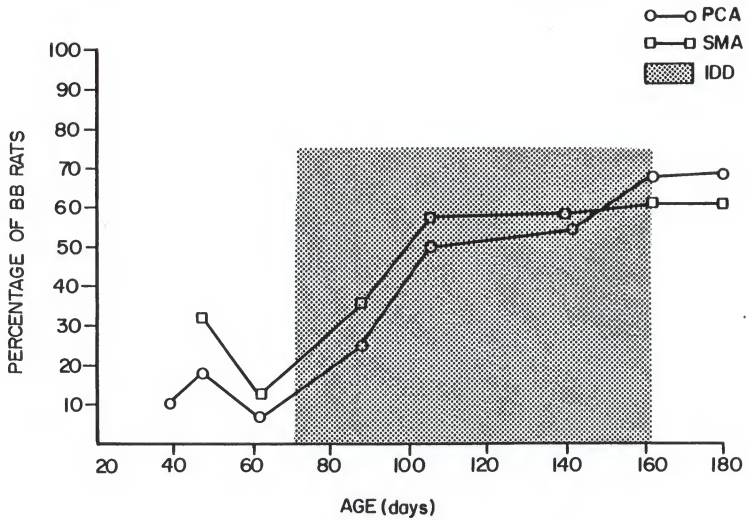
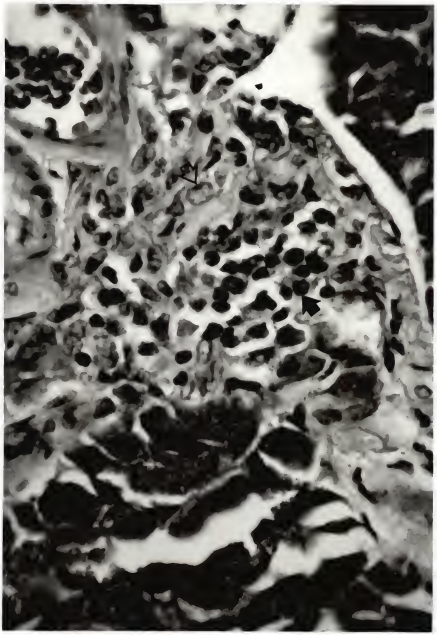

PCA
SMA
IDD
PERCENTAGE OF BB RATS
100
90
80
70
60
50
40
30
20
10
20
40
60
80
100
120
140
160
180
AGE (days)

FIGURE 3. Section of pancreas from a BB rat at onset of IDD. The islet in the center has disorganized architecture and lymphocytic infiltration (black arrow). A normal beta cell is indicated by a clear arrow.



control WF animals or BB x WF F1 rats, which were followed for at least 180 days after birth, developed IDD or pancreatic insulitis (Table 1), indicating that IDD in the BB rat is not due to a single dominant gene.

Presence of Organ-Specific Autoantibodies. ICA and other organ-specific autoantibodies were sought in BB/O and BB/W rats because these antibodies are characteristically found in human IDD, are evidence for an autoimmune etiology for IDD in the BB rat, and can be used as markers for animals with autoimmune tendencies. ICA were never detected in any BB rats, regardless of age or duration of IDD. To rule out the possibility that ICA were present but were directed against diabetic antigens, ICA were also sought on pancreatic sections from both nondiabetic BB rats and BB rats at onset of IDD. However, no such autoantibodies were identified. In addition, ICA were not demonstrated in BB rat sera when using fluorescein conjugated antirat Ig (all classes) instead of antirat IgG in case ICA of the IgM class were present. Thyroid microsomal autoantibodies and adrenal autoantibodies were never found in any BB animals.

However, autoantibodies to the parietal cells of the gastric mucosa (PCA) (Figure 4), thyroid colloid antigens and smooth muscle (SMA) (Figure 5) were demonstrated in the sera of a considerable number of BB/O and BB/W rats (Table 1). PCA were detected in the sera of 35% (7/20) of the BB/O rats and in the sera of of 68% (19/28) of the BB/W animals.

FIGURE 4. Positive indirect immunofluorescence staining of the parietal cells of rat gastric fundus.

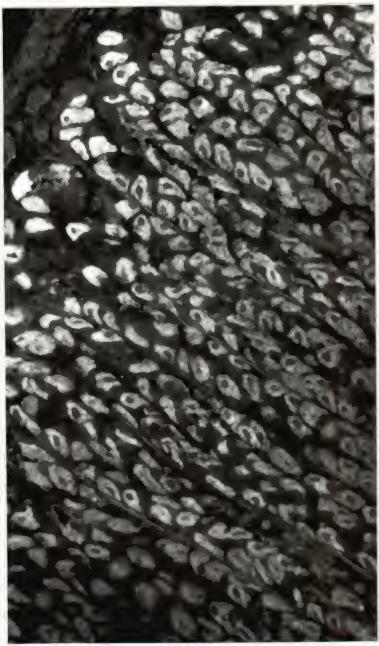
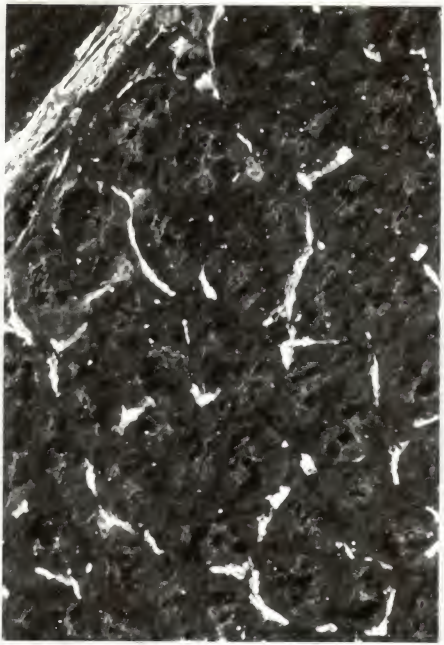


FIGURE 5. Positive indirect immunofluorescence staining of smooth muscle in rat gastric fundus.



The intensity of immunofluorescence of the PCA-positive sera tended to increase with duration of PCA. Except for an equivocal result from the serum of one WF rat, none of the WF or BB x WF F1 animals had demonstrable PCA in their serum. As was the case with IDD, PCA did not seem to have a dominant mode of inheritance. Thyroid colloid autoantibodies were of low immunofluorescence intensity and were less frequent than PCA, being found in only 5% (1/20) and 18% (5/28) of the sera from BB/O rats and BB/W rats, respectively. None of the control animals had autoantibodies to thyroid colloid. SMA were demonstrated in 55% (11/20) of BB/O rats, in 61% (17/28) of BB/W rats, and in 9% (7/80) of WF and BB x WF F1 animals. The presence of SMA was unrelated to the presence of IDD or PCA in the BB rats (Table 2).

Some 71% (5/7) of the BB/O rats with PCA and 79% (15/19) of the BB/W rats with PCA had IDD (Table 2). However, two PCA-positive BB animals (one BB/O and one BB/W) without clinical evidence of IDD had abnormal glucose tolerance tests as previously mentioned. Thus, only 15% (4/26) of the BB rats with detectable PCA had no discernible IDD confirmed by normal glucose tolerance tests.

The appearance of PCA in the serum usually shortly preceded the onset of IDD in BB rats that developed the disease. The frequency of PCA in the BB rats was seen to increase at an age coincident with the development of IDD and did not further rise after the critical age span for

TABLE 2

RELATIONSHIPS BETWEEN IDD, PCA AND SMA
IN BB/O, BB/W AND WF RATS

Rats Studied	Total	PCA %	SMA %
I. Total BB rats with IDD	37	54	57
Total BB rats without IDD	11	54	64
BB/O rats with IDD	16	31	50
BB/O rats without IDD	4	50	75
BB/W rats with IDD	21	71	62
BB/W rats without IDD	7	57	57
Rats Studied	**Total**	**IDD %**	**SMA %**
II. Total BB rats with PCA	26	77	46
Total BB rats without PCA	22	77	73
BB/O rats with PCA	7	71	57
BB/O rats without PCA	13	85	54
BB/W rats with PCA	19	79	42
BB/W rats without PCA	9	67	100

onset of IDD in these rats had passed (Figures 1 and 2). Although BB/W rats were susceptible to IDD for a longer age range than BB/O animals, the marked increase in the frequency of PCA in BB/W rats between 70 and 110 days of age mirrored the increase in PCA frequency observed in BB/O rats between 90 and 113 days of age. The frequency of SMA in BB rats increased with age and, unlike PCA, did not closely parallel the development of IDD in BB/O rats. The frequency of SMA, however, increased in parallel with PCA in the BB/W animals.

Functional abnormalities of the gastric parietal cells were sought in BB rats with PCA as evidence that gastric autoimmunity may result in clinical disease. Achlorhydria was not demonstrated in the gastric aspirates of BB rats with PCA, the pH values of which ranged from 2 to 3. Vitamin B12 levels were measured in sera from 14 BB rats with PCA, 14 PCA-negative BB rats, and 7 control WF rats. No statistically significant differences were seen in serum vitamin B12 levels between the three groups as evaluated by Student's t-test (Table 3). Comparison of serum iron levels and total iron binding capacities between 14 BB rats with PCA, 11 BB rats without PCA and 7 control WF animals also revealed no significant differences (Table 3).

Histological examinations of stomach sections from 12 BB rats with PCA were performed in order to see if lymphocytic infiltration suggestive of autoimmunity were present. All sections revealed mild to moderate lymphocytic

TABLE 3

SERUM VITAMIN B12 AND IRON LEVELS IN BB AND WF RATS

	BB rats with PCA (n=14)[a]	BB rats without PCA[a] (n=14)	WF rats (n=7)
Vitamin B12	$\bar{x}$ = 472 ± 38 (244-710)	$\bar{x}$ = 466 ± 44 (109-759)	$\bar{x}$ = 473 ± 49 (275-666)
	BB rats with PCA (n=14)	**BB rats without PCA (n=11)**	**WF rats (n=7)**
Iron	$\bar{x}$ = 223 ± 13 (84-352)	$\bar{x}$ = 210 ± 21 (131-326)[b]	$\bar{x}$ = 234 ± 33 (84-372)
Total iron binding capacity	$\bar{x}$ = 492 ± 17 (328-620)	$\bar{x}$ = 474 ± 36 (355-565)	$\bar{x}$ = 519 ± 78 (285-742)
% iron binding	$\bar{x}$ = 45 ± 2 (33- 78)	$\bar{x}$ = 48 ± 5 (35- 60)	$\bar{x}$ = 51 ± 6 (26- 70)

[a] The BB rats used are from the group of 48 animals designated as either BB/O (20 rats) or as BB/W (28 rats). All results are expressed as the mean ± 1 standard error of the mean.

[b] Sera from 11 BB rats without PCA were used to determine serum iron levels, however only sera from 9 BB rats without PCA had measurements for iron binding capacity and percent iron binding performed.

infiltrations of the gastric mucosa with some loss of normal mucosal cells and increased fibrosis (Figure 7), in comparison with gastric fundus obtained from WF and PCA-negative BB rats (Figure 6). However, severe atrophy of the gastric mucosa was not found in any of the tissues studied. In two BB rats with PCA for the longest periods of approximately 7 months, degrees of squamous metaplasia of the gastric mucosa were seen in sections taken well below the junction between the proximal stomach and the fundus (Figure 8). Only one of these rats had IDD. No stomach sections from spleen BB rats without PCA or 5 control WF rats revealed inflammatory lesions of the gastric mucosa.

Characterization of Peripheral Leukocyte Populations. Due to observations of increased susceptibility to opportunistic infections (especially of the respiratory tract) in both diabetic and nondiabetic BB rats, and indications of both pancreatic and gastric autoimmunities in this strain, immunological studies of these animals were performed.

Increased percentages and absolute numbers of peripheral blood polymorphonuclear leukocytes (PMNs) were observed in all BB rats regardless of the presence of IDD, in comparison with WF and F1 hybrid rats ($p < 0.0025$, Table 4), perhaps reflective of the increased rate of infections among these animals. In contrast, all 16 BB rats with IDD and all 32 nondiabetic rats, ranging in age from 25 to 400 days, were observed to have significantly decreased absolute

FIGURE 6. Section of normal BB rat gastric fundus stained with hematoxylin and eosin. Organized rows of parietal cells (black arrow) are present.

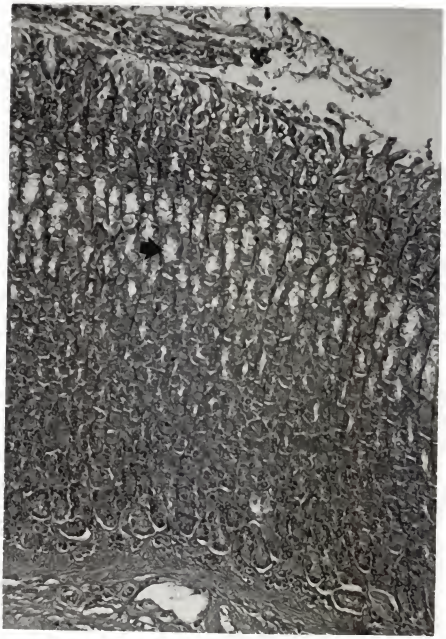


FIGURE 7. Hematoxylin and eosin stained section of gastric fundus from a BB rat with PCA showing lymphocytic infiltration of the mucosa (black arrow).

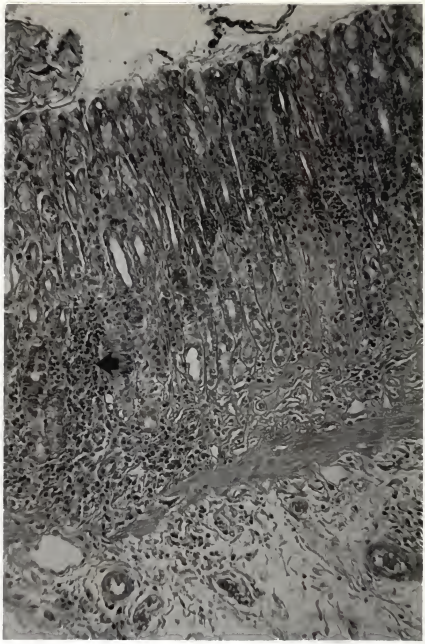


FIGURE 8. Hematoxylin and eosin stained section of gastric fundus from a BB rat with PCA for 7 months. Lymphocytic infiltration, fibrosis and squamous metaplasia (black arrow) of the mucosa are present.

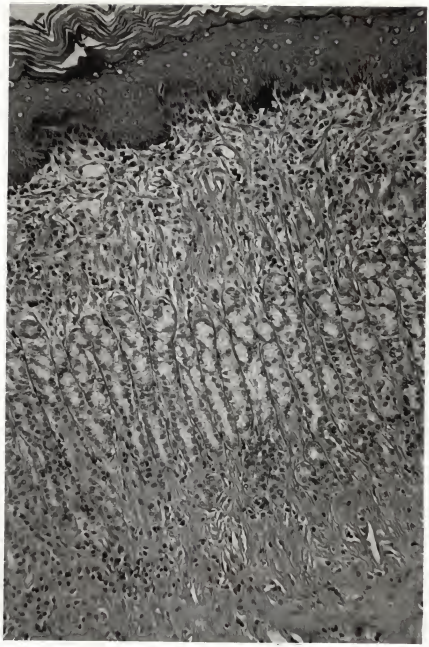


TABLE 4

ENUMERATION OF PERIPHERAL BLOOD LEUKOCYTES IN BB, WF AND BB x WF F1 RATS

	Animals Studied			
	BB Rats With IDD (n=16)	NonDiabetic BB Rats (n=32)	WF Rats (n=28)	F1 Rats (n=8)
Leukocytes/mm^3	5673 ± 1840[a,b]	5655 ± 1761[b]	9680 ± 2858	10843 ± 1521
PMNs/mm^3	2770 ± 1132[c]	2225 ± 1015[c]	1334 ± 923	1275 ± 503
%PMNs	57 ± 11[c]	34 ± 11[c]	14 ± 7	12 ± 3
Lymphocytes/mm^3	2805 ± 1071[c,e]	3403 ± 1041[b]	7740 ± 2317	9105 ± 1084
% Lymphocytes	47 ± 15[c,d]	60 ± 12[b]	82 ± 7	84 ± 4
Monocytes/mm^3	228 ± 140	187 ± 113	277 ± 201	432 ± 251
% Monocytes	4 ± 4	4 ± 2	3 ± 1	4 ± 2
Eosinophils/mm^3	46 ± 45	136 ± 309	31 ± 58	0
% Eosinophils	2 ± 4	2 ± 4	1 ± 3	0

Table 4--extended.

[a] Each value is stated as mean ± S.D.

[b] $p < 0.0005$ by Student's t test when compared to WF and F1 rats.

[c] $p < 0.0025$ when compared to WF and F1 rats.

[d] $p < 0.0025$ when compared to BB rats without IDD.

[e] $p < 0.01$ when compared to BB rats without IDD.

numbers of both total peripheral blood leukocytes and lymphocytes, when compared to 28 control WF rats and 8 F1 hybrid animals ($p < 0.0005$). The lymphopenia was more striking than the leukopenia, reflecting significant complementary decreases in the percentage of lymphocytes seen in BB rats from that observed in WF and BB x WF F1 rats ($p < 0.0005$). Significant differences were also seen in both the percentages and absolute numbers of lymphocytes in diabetic BB rats when compared to BB rats without IDD ($p < 0.01$).

Due to the severe lymphopenia observed in all BB rats, analyses of lymphocyte subpopulations were next performed. Irrespective of age or the presence of IDD, increased percentages ($p < 0.0005$) but similar absolute numbers of Ia-positive cells (MRC OX6 monoclonal antibody reactive) and Ig-positive cells were found in BB rats in comparison with WF rats and BB x WF F1 rats (Table 5). In contrast, the absolute numbers of peripheral T lymphocytes (W3/13 monoclonal antibody reactive) were significantly lower in all BB rats when compared to WF and F1 animals ($p < 0.001$). Correspondingly significant depressions of both absolute numbers ($p < 0.005$) and percentages ($p < 0.025$) of W3/25-positive cells (helper T cells) were observed in all BB rats, independent of age or IDD, in comparison with WF and F1 animals. Increased percentages ($p < 0.005$) but decreased absolute numbers ($p < 0.005$) of cytotoxic/ suppressor T lymphocytes (MRC OX8 monoclonal antibody reactive) were also

TABLE 5

LYMPHOCYTE SUBSETS IN BB, WF AND BB x WF F1 RATS

	Animals Studied			
	BB Rats With IDD (n=8)	BB Rats Without IDD (n=15)	WF Rats (n=12)	F1 Rats (n=4)
Leukocytes/mm^3	4837 ± 2373[a,b]	4921 ± 1754[b]	10154 ± 3858	9475 ± 2306
Lymphocytes/mm^3	2986 ± 991[b]	2847 ± 943[b]	8296 ± 2714	7441 ± 1840
$W3/13^+$ Cells/mm^3	2060 ± 817[c]	2069 ± 818[c]	6034 ± 1421	5809 ± 1051
% $W3/13^+$ Cells	70 ± 14	71 ± 10	74 ± 9	79 ± 8
$W3/25^+$ Cells/mm^3	1323 ± 688[c]	1097 ± 501[c]	4243 ± 1035	3840 ± 1343
% $W3/25^+$ Cells	37 ± 15[c]	38 ± 13[d]	50 ± 9	47.5 ± 2
MRC $OX8^+$ Cells/mm^3	1650 ± 729[c]	1258 ± 505[c]	3431 ± 902	3358 ± 1327
% MRC $OX8^+$ Cells	46.5 ± 9[d]	44 ± 6[e]	38 ± 7	41 ± 2
MRC $OX6^+$ Cells/mm^3	1351 ± 462	1377 ± 460	1592 ± 649	1260 ± 247
% MRC $OX6^+$ Cells	43.5 ± 15[b]	47 ± 12[b]	18 ± 4	16.5 ± 2

Table 5--extended.

Ig^+ Cells/mm^3	1158 ± 446	1384 ± 559	1324 ± 509	1370 ± 899
% Ig^+ Cells	36.5 ± 10[b]	47 ± 14[b]	16 ± 4	17 ± 8

[a] Each value is stated as mean ± S.D.

[b] $p < 0.0005$ by Student's t test when compared to WF rats.

[c] $p < 0.005$ by Student's t test when compared to WF rats.

[d] $p < 0.025$ by Student's t test when compared to WF rats.

[e] $p < 0.01$ by Student's t test when compared to WF rats.

observed in all BB rats when compared to WF control and BB x WF F1 rats. Numbers of T lymphocytes in both lymphocyte subsets of F1 hybrid animals tended to range between those of WF and BB rats. However, there were no significant differences between WF and BB x WF F1 values.

An inversion of the W3/25-positive subset to MRC OX8-positive subset ratio to less than 1.0 (mean 0.7 $\pm$ 0.2) also occurred in BB rats between 75 to 115 days of age, which was not influenced by the presence of IDD ($p < 0.001$, Figure 9). In younger BB rats with and without IDD, the mean W3/25-positive subset to MRC OX8-positive subset ratio was similar to the mean ratio seen in WF rats at all ages studied (1.2 $\pm$ 0.2 versus 1.3 $\pm$ 0.1).

Presence of Thymocytotoxic Autoantibodies. As a possible explanation for the extremely decreased numbers of T lymphocytes present in BB rats, autoantibodies to BB or WF thymocytes were sought in these animals. As shown in Table 6, many unabsorbed sera from both diabetic and nondiabetic BB rats had demonstrable thymocytotoxic autoantibodies, especially when the sera were tested at 1:2 dilution. Although a few WF sera were also antibody positive, significantly more BB sera had antibodies to thymocytes ($p < 0.05$). Only sera giving reactions of greater than 50% cytotoxicity were considered to be positive for these autoantibodies.

Depressed Ability to Reject Allografts. Because BB rats had both severely decreased numbers of peripheral T lympho-

FIGURE 9. A plot of the ratio of the W3/25-positive subset (helper T lymphocytes) to the MRC OX8-positive subset (cytotoxic/ suppressor T cells) versus age of the BB rats. N designates nondiabetic BB rats and D designates BB rats with IDD.

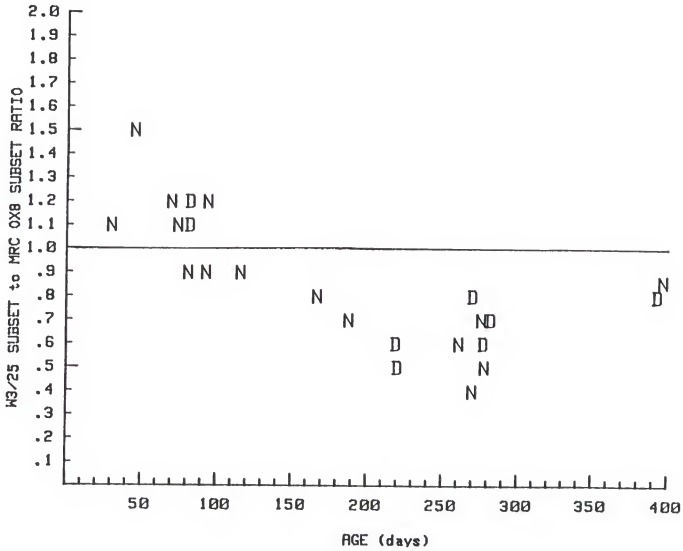

W3/25 SUBSET to MRC OX8 SUBSET RATIO
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
.9
.8
.7
.6
.5
.4
.3
.2
.1
50
100
150
200
250
300
350
400
AGE (days)

TABLE 6

THYMOCYTOTOXIC AUTOANTIBODIES IN BB AND WF RAT SERA

Sera		Autoantibody Positive
BB rats with IDD	undiluted	25% (16/65)
	1:2 dilution	32% (10/31)[b]
BB rats without IDD	undiluted	16% (6/38)
	1:2 dilution	47% (8/17)[a]
WF rats	undiluted	6% (1/18)
	1:2 dilution	9% (2/23)

[a] $p < 0.05$ by Chi-square analysis when compared to WF rats.

[b] $p < 0.01$ when compared to WF rats.

cytes and circulating thymocytotoxic autoantibodies, the ability of T lymphocytes from BB rats to function normally in vivo was studied. Both BB and WF rats are thought to share the rat major histocompatibility complex RT.1^u genotype, while Lewis rats have the RT.1^l haplotype (106-111). Lewis skin grafts would thus be expected to be rejected by both BB and WF rats in less than 14 days. However, as seen in Table 7, nondiabetic BB rats rejected Lewis allografts significantly more slowly than expected and in comparison with WF controls ($p < 0.0005$). Furthermore, WF skin grafts were not rejected by nondiabetic BB rats, while rejections of BB allografts by WF rats occurred in 17 ± 3 days ($p < 0.0005$), suggesting the presence of multiple minor histocompatibility differences between BB and WF rats. BB rats were thus extremely deficient in their ability to reject grafts across both major and minor histocompatibility barriers, and this defect was not dependent on the presence of IDD.

Mitogen Responsiveness. Since the skin graft results suggested that BB rats have defective in vivo T cell-mediated immune responses, the ability of T lymphocytes from BB rats to respond to mitogens in vitro was next studied. PWM, PHA and Con A, which are primarily T cell mitogens in the rat (112-114), were used. Dose-response curves were initially prepared for each mitogen using varying concentrations of each mitogen and of WF spleen cells ($0.3\text{-}2 \times 10^5$

TABLE 7

ALLOGRAFT REJECTION BY BB AND WF RATS

Recipients	Donor	Graft Survival (Days)[a]
8 BB rats without IDD	Lewis	30 ± 4
6 WF rats	Lewis	12 ± 2
6 BB rats without IDD	WF	> 90
6 WF rats	BB	17 ± 3

[a] The period of graft survival was measured from day of graft placement to day of complete graft rejection. Each value is expressed as mean ± S.D.

cells/well) as responders (Figures 10-12). Optimal stimulation indices were obtained from these curves and subsequent experiments using 1.0 μg/ml and 5.0 μg/ml PWM, 0.5% and 1.0% PHA, and 1.0 μg/ml and 5.0 μg/ml Con A in each well. WF cells responded well to these mitogens at all cell concentrations tested, but subsequent response comparisons with BB cells were made using spleen cell or PBL concentrations at 0.3×10^5, 1×10^5 and 2×10^5 cells/well. The results were not dependent on the BB cell concentrations used or on the presence of IDD in the BB rats.

Utilizing spleen cell concentrations of 0.3×10^5 cells/well (Figure 13), 1×10^5 cells/well (Figure 14), and 2×10^5 cells/well ($p < 0.0005$, Figure 15), BB splenic lymphocytes showed markedly diminutive responses to PWM in comparison to WF splenic lymphocytes at the same mitogen concentration. Similar relative results were obtained when using 0.3×10^5 PBL/well (Figure 16) or 1×10^5 PBL/well ($p < 0.01$, Figure 17). However, the level of proliferation of WF lymphocytes to PWM using 0.3×10^5 PBL/well was quite low, suggesting that insufficient numbers and/or types of cells were present for optimal proliferation. Both BB and WF proliferative responses to PWM (also PHA and Con A) were higher using spleen cells rather than PBL as responders but background counts also tended to be higher. PWM responses of BB lymphocytes did increase with increasing cell numbers per well as expected. However, BB results comparable to those by WF cells were only observed when

FIGURE 10. Proliferative responses (cpm) of increasing concentrations of WF splenic lymphocytes to 3 concentrations of PWM measured on day 3. The following spleen cell concentrations were used: 0.2×10^5 cells/well (open circle), 0.4×10^5 cells/well (open triangle), 0.6×10^5 cells/well (open square), 0.8×10^5 cells/well (open hexagon), 1.0×10^5 cells/well (closed circle), 1.2×10^5 cells/well (closed triangle) and 1.6×10^5 cells/well (closed square).

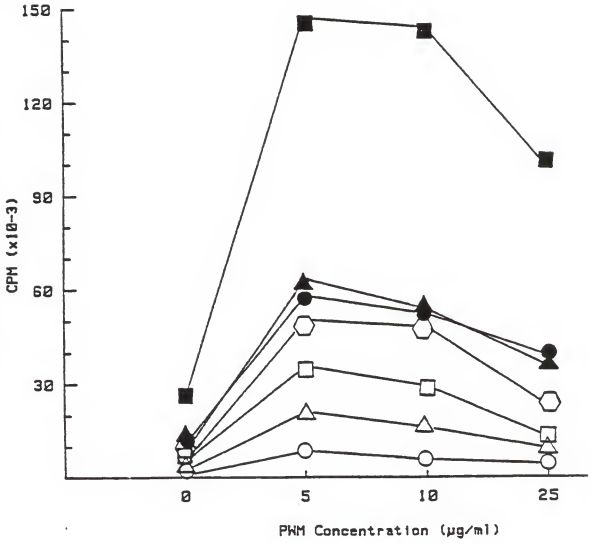

150
120
90
60
30
CPM (x10-3)
0
5
10
25
PWM Concentration (µg/ml)

FIGURE 11. Proliferative responses (cpm) of increasing concentrations of WF splenic lymphocytes to 5 concentrations of PHA measured after 3 days. These spleen cell concentrations were used: 0.2×10^5 cells/well (open circle), 0.4×10^5 cells/well (closed circle), 0.6×10^5 cells/well (open triangle), 0.8×10^5 cells/well (closed triangle), 1.0×10^5 cells/well (open square), 1.2×10^5 cells/well (closed square) and 1.6×10^5 cells/well (open hexagon).

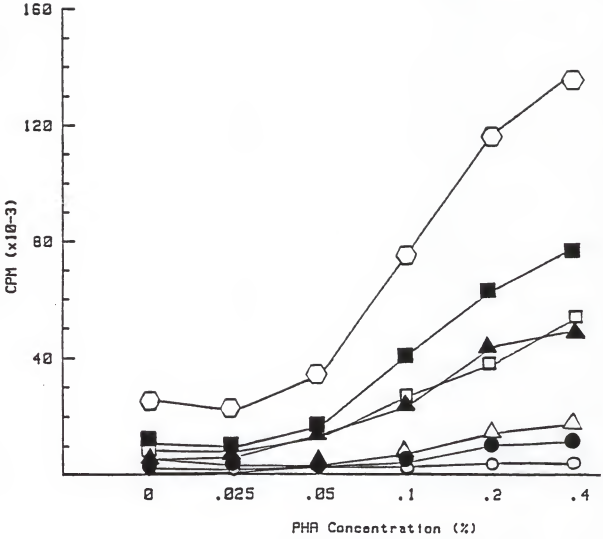

160
120
80
40
CPM (x10-3)
0
.025
.05
.1
.2
.4
PHA Concentration (%)

FIGURE 12. Proliferative responses (cpm) of increasing concentrations of WF splenic lymphocytes to 3 concentrations of Con A measured on day 3. The following spleen cell concentrations were used: 0.2×10^5 cells/well (open circle), 0.4×10^5 cells/well (open triangle), 0.6×10^5 cells/well (open square), 0.8×10^5 cells/well (open hexagon), 1.0×10^5 cells/well (closed circle), 1.2×10^5 cells/well (closed triangle) and 1.6×10^5 cells/well (closed square).

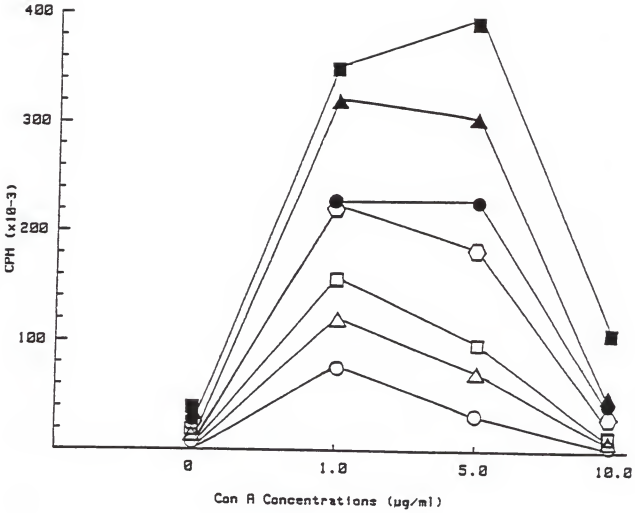

400
300
200
100
CPM (x10-3)
0
1.0
5.0
10.0
Con A Concentrations (µg/ml)

FIGURE 13. Responses (cpm) of splenic lymphocytes at 0.3 x 10^5 cells/well from 2 nondiabetic BB rats (striped bars) and 2 WF rats (open bars) to 1 μg/ml PWM and 4 μg/ml PWM. Each value is mean of triplicate cultures ± S.D.

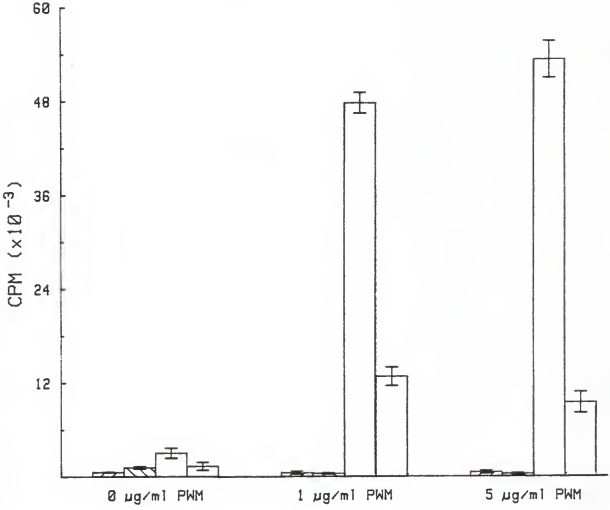

60
48
36
24
12
CPM (x10^{-3})
0 µg/ml PWM
1 µg/ml PWM
5 µg/ml PWM

FIGURE 14. Proliferative responses (cpm) of splenic lymphocytes at 1 x 10^5 cells/well from 2 nondiabetic BB rats (striped bars) and 3 WF rats (open bars) to 2 concentrations of PWM. Each value is mean of triplicate cultures $\pm$ S.D.

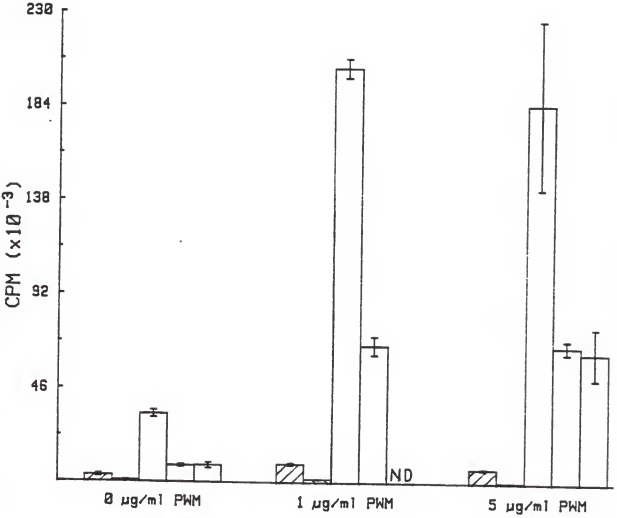

230
184
138
92
46
CPM (x10^{-3})
ND
0 µg/ml PWM
1 µg/ml PWM
5 µg/ml PWM

FIGURE 15. Responses (cpm) of splenic lymphocytes at 2 x 10^5 cells/well from 4 nondiabetic BB rats (striped bars) and 4 WF rats (open bars) to 10 μg/ml PWM. Each value is mean of triplicate cultures ± S.D.

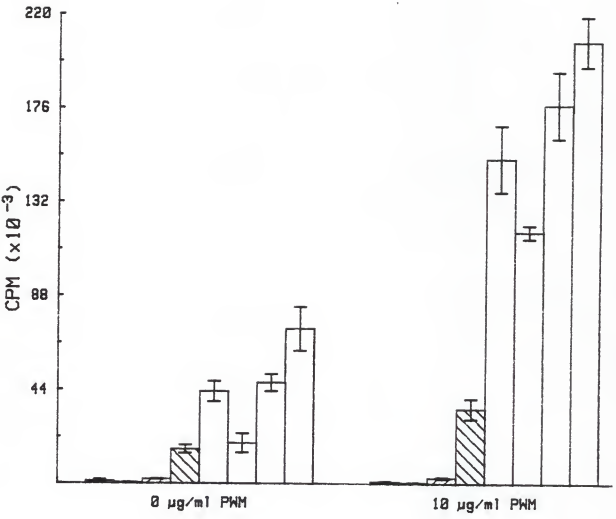

220
176
132
88
44
CPM (x10⁻³)
0 µg/ml PWM
10 µg/ml PWM

FIGURE 16. Responses (cpm) of PBL at 0.3 x 10^5 cells/well from 1 diabetic BB rat (first striped bar), 2 nondiabetic BB rats (remaining striped bars) and 3 WF rats (open bars) to 1 μg/ml PWM and 5 μg/ml PWM. Each value is mean of triplicate cultures ± S.D.

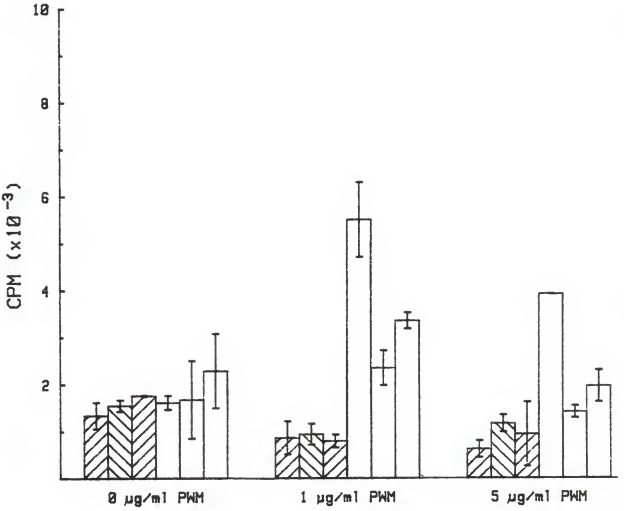
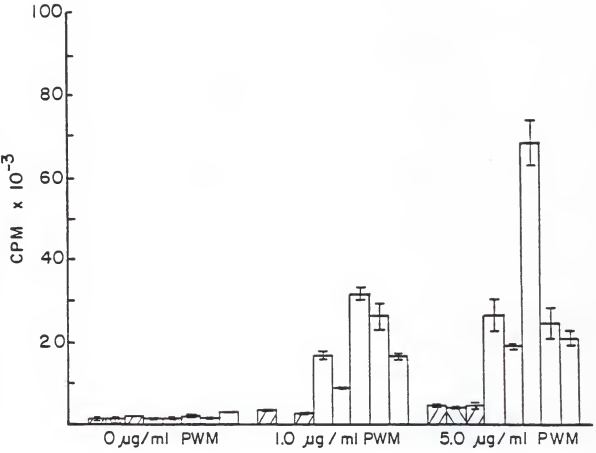

10
8
6
4
2
CPM (x10 -3)
0 µg/ml PWM
1 µg/ml PWM
5 µg/ml PWM

FIGURE 17. PWM responses (cpm) of PBL at 1.0 x 10^5 cells/well from 3 nondiabetic BB rats (striped bars) and 5 WF rats (open bars). Each value is mean of triplicate cultures $\pm$ S.D.


100
80
60
40
20
CPM x 10^{-3}
0 µg/ml PWM
1.0 µg/ml PWM
5.0 µg/ml PWM

using 2 x 10^5 BB spleen cells/well and 0.3 x 10^5 WF spleen cells/well.

Lymphocytes from BB rats also responded poorly to various concentrations of PHA at 0.3 x 10^5 spleen cells/well (Figure 18), 1 x 10^5 spleen cells/well (Figure 19), and 2 x 10^5 spleen cells/well ($p < 0.05$, Figure 20), in comparison with WF lymphocytes. Although similar results were obtained when using 1 x 10^5 PBL/well ($p < 0.01$, Figure 21), low levels of proliferation with no significant differences between WF and BB PHA responses at 0.3 x 10^5 PBL/well (Figure 22) were noted, most likely due to insufficient numbers of cells as stated previously.

Although lymphocytes from BB rats were most responsive to Con A of the mitogens used, WF cells still responded significantly better at all of the following cell concentrations: 0.3 x 10^5 spleen cells/well (Figure 23), 1 x 10^5 spleen cells/well (Figure 24), 1 x 10^5 PBL/well ($p < 0.01$, Figure 25) and 2 x 10^5 spleen cells/well ($p < 0.005$, Figure 26). As was true for PWM and PHA, WF lymphocytes at 0.3 x 10^5 PBL/well responded poorly to Con A and thus no significant differences were observed at this cell concentration between BB and WF proliferative responses (Figure 27). However, in one experiment, splenic lymphocytes from a single BB rat at 2 x 10^5 cells/well were able to mount a significant response to Con A comparable to responses by WF spleen cells. However, BB lymphocytes from the same animal minimally responded to Con

FIGURE 18. PHA responses (cpm) of splenic lymphocytes at 0.3×10^5 cells/well from 2 nondiabetic BB rats (striped bars) and 2 WF rats (open bars). Each value is mean of triplicate cultures $\pm$ S.D.

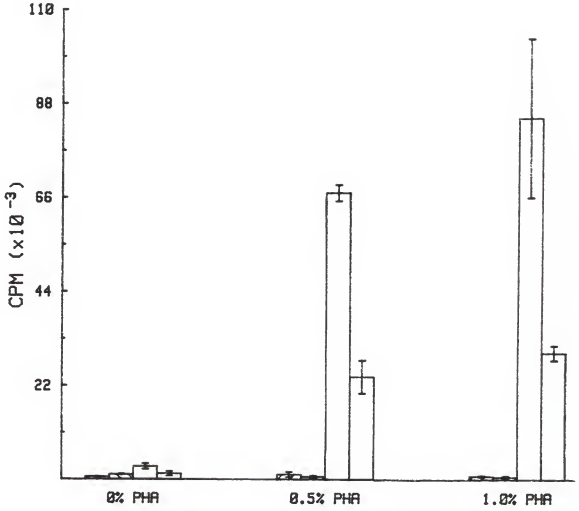

110
88
66
44
22
CPM (x10⁻³)
0% PHA
0.5% PHA
1.0% PHA

FIGURE 19. Proliferative responses (cpm) of splenic lymphocytes at 1 x 10^5 cells/well from 2 nondiabetic BB rats (striped bars) and 2 WF rats (open bars) to 0.5% and 1.0% PHA. Each value is mean of triplicate cultures $\pm$ S.D.

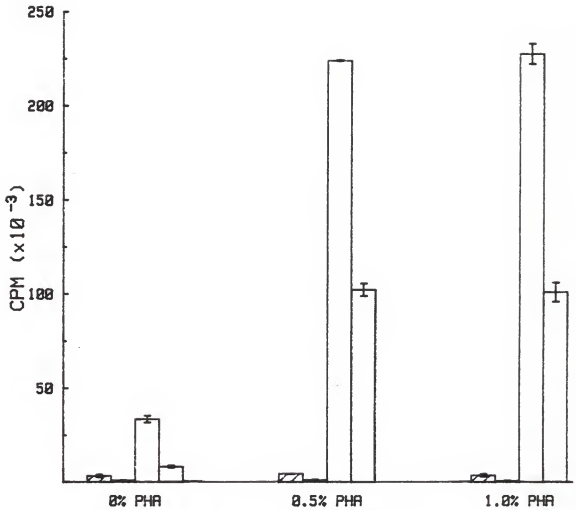

250
200
150
100
50
CPM (x10^{-3})
0% PHA
0.5% PHA
1.0% PHA

FIGURE 20. Comparison of proliferative responses (cpm) of splenic lymphocytes at 2 x 10^5 cells/well from 2 non-diabetic BB rats (striped bars) and 2 WF rats (open bars) to 5 concentrations of PHA. Each value is mean of triplicate cultures $\pm$ S.D.

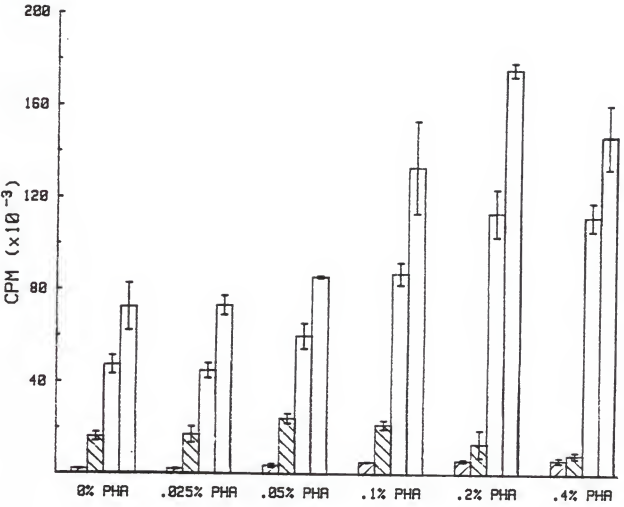

200
160
120
80
40
CPM (x10^{-3})
0% PHA
.025% PHA
.05% PHA
.1% PHA
.2% PHA
.4% PHA

FIGURE 21. Proliferative responses (cpm) of PBL at 1 x 10^5 cells/ well from 1 diabetic BB rat (first striped bar), 2 nondiabetic BB rats (remaining striped bars) and 5 WF rats (open bars) to 2 PHA concentrations. Each value is mean of triplicate cultures $\pm$ S.D.

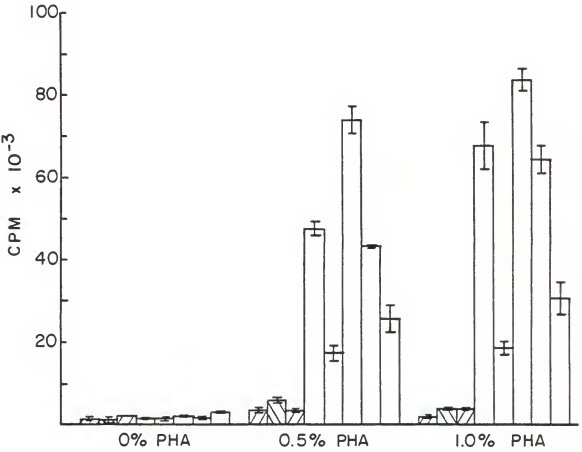

100
80
60
40
20
CPM x 10-3
0% PHA
0.5% PHA
1.0% PHA

FIGURE 22. PHA responses (cpm) of PBL at 0.3 x 10^5 cells/well from 1 diabetic BB rat (first striped bar), 2 non-diabetic BB rats (remaining striped bars) and 3 WF rats (open bars). Each value is mean of triplicate cultures $\pm$ S.D.

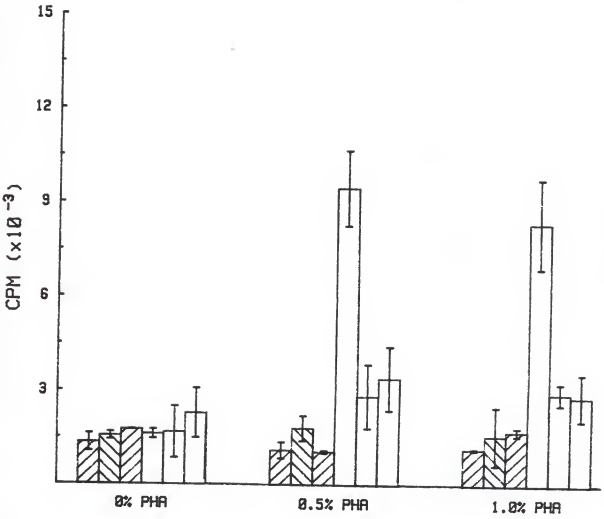
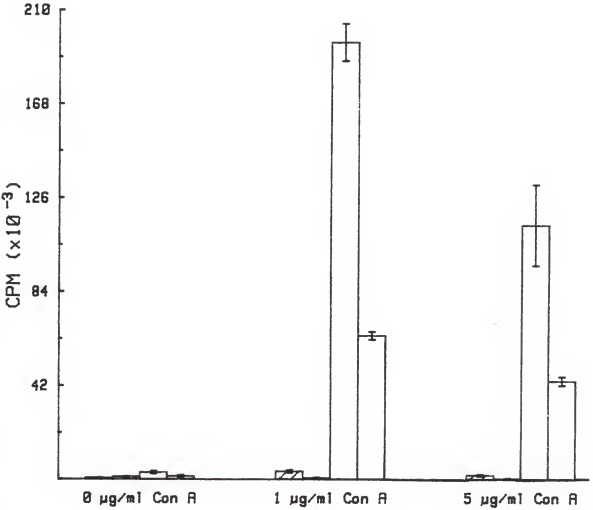

15
12
9
6
3
CPM (x10⁻³)
0% PHA
0.5% PHA
1.0% PHA

FIGURE 23. Con A responses of splenic lymphocytes at 0.3 x 10^5 cells/well from 2 nondiabetic BB rats (striped bars) and 2 WF rats (open bars). Each value is mean of triplicate cultures $\pm$ S.D.


210
168
126
84
42
CPM (x10 -3)
0 µg/ml Con A
1 µg/ml Con A
5 µg/ml Con A

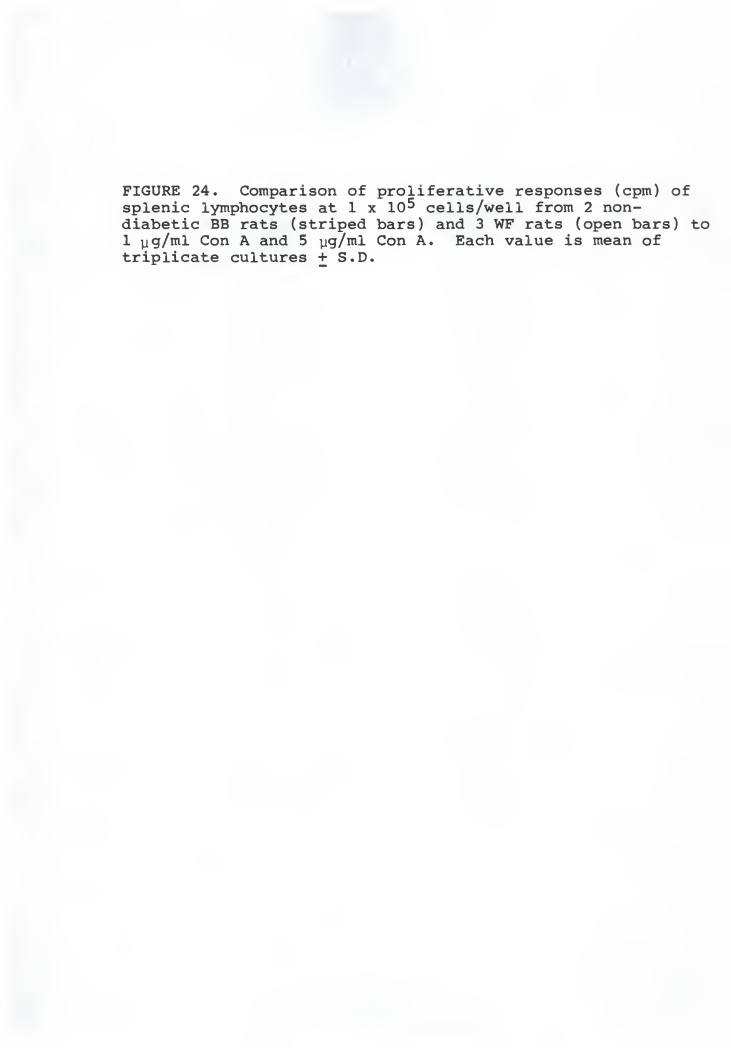


FIGURE 24. Comparison of proliferative responses (cpm) of splenic lymphocytes at 1 x 10^5 cells/well from 2 non-diabetic BB rats (striped bars) and 3 WF rats (open bars) to 1 μg/ml Con A and 5 μg/ml Con A. Each value is mean of triplicate cultures ± S.D.

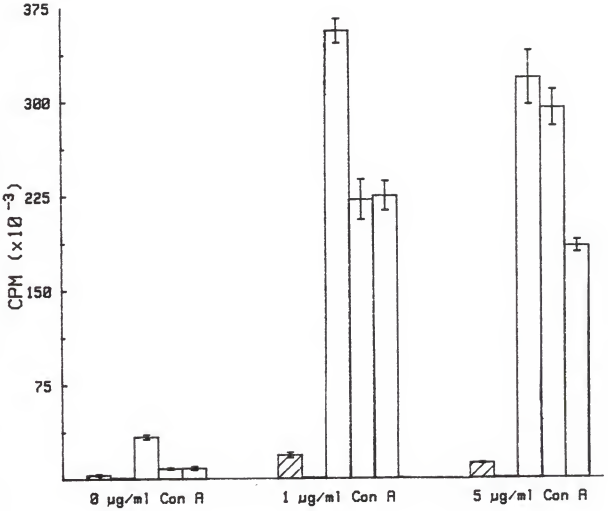

375
300
225
150
75
CPM (x10 -3)
0 µg/ml Con A
1 µg/ml Con A
5 µg/ml Con A

FIGURE 25. Proliferative responses (cpm) of PBL at 1 x 10^5 cells/well from 1 diabetic BB rat (first striped bar), 3 nondiabetic rats (remaining striped bars) and 5 WF rats (open bars) to 2 Con A concentrations. Each value is mean of triplicate cultures $\pm$ S.D.

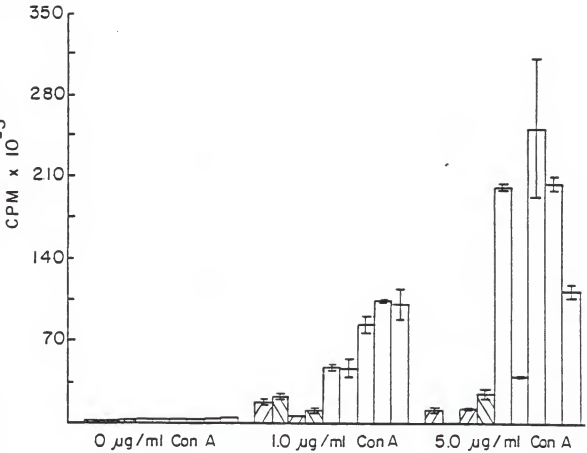

350
280
210
140
70
CPM x 10^{-3}
0 µg/ml Con A
1.0 µg/ml Con A
5.0 µg/ml Con A

FIGURE 26. Responses (cpm) of splenic lymphocytes at 2 x 10^5 cells/well from 4 nondiabetic BB rats (striped bars) and 4 WF rats (open bars) to 5 μg/ml Con A. Each value is mean of triplicate cultures ± S.D.

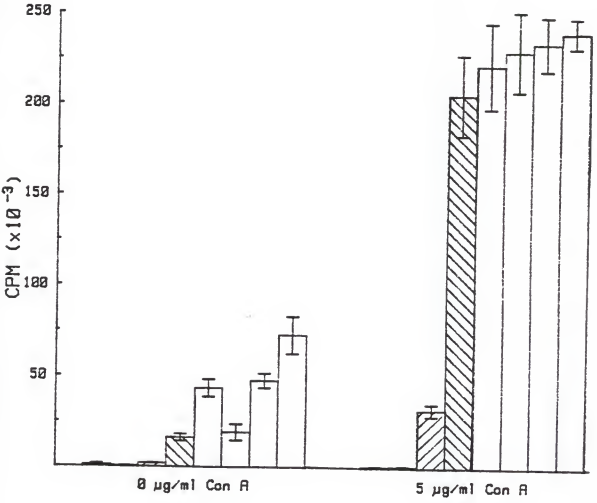
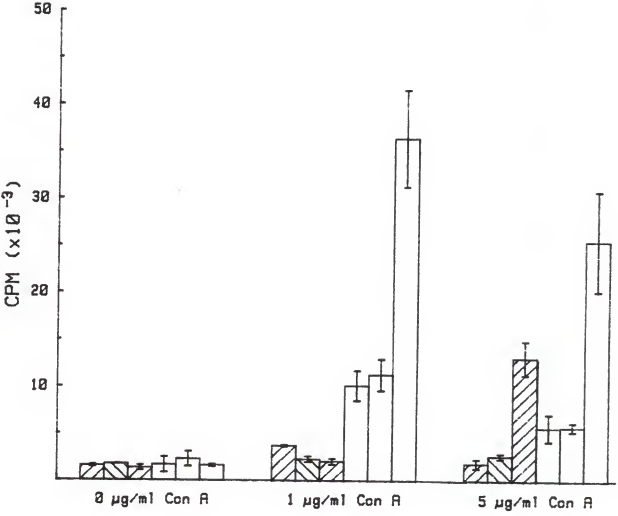

250
200
150
100
50
CPM (x10 -3)
0 µg/ml Con A
5 µg/ml Con A

FIGURE 27. Comparison of responses (cpm) of PBL at 0.3 x 10^5 cells/well from diabetic BB rat (first striped bar), 2 nondiabetic BB rats (remaining striped bars) and 3 WF rats (open bars) to 1 μg/ml Con A and 5 μg/ml Con A. Each value is mean of triplicate cultures ± S.D.


50
40
30
20
10
CPM (x10 -3)
0 µg/ml Con A
1 µg/ml Con A
5 µg/ml Con A

A at lower cell concentrations. Although sharp decreases in responsiveness to PWM, PHA and Con A were observed in many older WF rats who were greater than 250 days of age, no BB responses exceeded even the proliferative responses observed in older WF animals.

Effect of Irradiated or Nonirradiated Allogeneic Cells on Mitogenic Responses.

In order to determine whether the lack of significant responses by BB lymphocytes to mitogens was due to increased suppressor activity, irradiated or nonirradiated BB spleen cells were added to WF responders, and the subsequent mitogenic responses measured. To examine the possibility of insufficient levels of necessary lymphokines or helper factors as a cause of BB unresponsiveness, the effects of the addition of irradiated WF cells on BB mitogenic responses were also studied. Irradiated WF cells, although unable to proliferate, can secrete helper factors such as IL 2 (115,116). The presence of IL 2 or T cell growth factor is necessary for lymphocytes to proliferate in response to mitogens or to allogeneic cells in MLCs (117-120).

Experiments were performed with similar results using either PWM, PHA or Con A and either 1×10^5 PBL/well or 0.5×10^5 spleen cells/well as responders. Proliferation of WF splenic lymphocytes to varying concentrations of Con A (0.5 - 4.0 μg/ml) was not overall significantly suppressed by the addition of either 0.5×10^5 irradiated or 0.5×105 nonirradiated BB spleen cells, nor were the

mitogenic responses of BB lymphocytes enhanced by the addition of 0.5 x 10^5 irradiated WF spleen cells (Table 8). These latter results were, however, somewhat equivocal about the inhibitory effects of added BB cells on WF responses. Similar findings were obtained with PWM and PHA using 0.5 x 10^5 spleen cells/well (Table 9), and with PWM and Con A at 1.0 x 10^5 PBL/well (Table 10). Thus, the inability of BB lymphocytes to respond to mitogenic stimulation does not seem to be solely related to either increased suppressor activity or to deficient levels of lymphokines necessary for proliferation in BB reactions.

Lack of MLC Responses. Proliferative responses by lymphocytes from BB rats to allogeneic cells in MLCs were also studied for comparison with the skin graft findings. Initially, the responses of 0.5 x 10^5 unseparated spleen cells from BB and WF rats to 1 x 10^5 and 2 x 10^5 irradiated Lewis stimulators were compared. As revealed in three representative experiments presented in Table 11, lymphocytes from BB rats were unable to proliferate to allogeneic Lewis spleen cells in MLCs, while WF lymphocytes responded well to Lewis stimulator cells. The addition of IL 2-containing WF Con A sups or irradiated WF spleen cells did not enhance the allogeneic responses of BB lymphocytes to Lewis stimulators. Thus, these findings as well as the mitogen results, suggest that even in the presence of helper factors such as IL 2, BB lymphocytes cannot mount immune

TABLE 8

I. EFFECT OF ADDITION OF IRRADIATED OR NONIRRADIATED ALLOGENEIC CELLS ON MITOGENIC RESPONSES OF BB AND WF SPLEEN CELLS

Responder (cells/well)	Con A responses (cpm)[b] Concentrations of Con A (μg/ml)				
	0	0.5	1.0	2.0	4.0
A = BB 0.5×10^5	1455 ± 242[a]	17481 ± 1480	31858 ± 611	30697 ± 2631	29317 ± 1506
B = WF 0.5×10^5	15459 ± 485	202133 ± 829	272274 ± 17598	327044 ± 7002	210640 ± 97
A + Bx[c] 0.5×10^5 each	6511 ± 2591	16512 ± 4095	20701 ± 661	18479 ± 1342	14751 ± 2398
A + B 0.5×10^5 each	21971 ± 2106	180971 ± 1114	266064 ± 11759	294636 ± 7358	246163 ± 20079
B + Ax 0.5×10^5 each	19024 ± 13958	144299 ± 7819	254043 ± 13185	306300 ± 21026	302577 ± 2361
Ax 0.5×10^5	4673 ± 366	7232 ± 17	7557 ± 527	602 ± 5	1492 ± 133
Bx 0.5×10^5	1655 ± 953	795 ± 232	804 ± 171	1340 ± 1290	3726 ± 266

[a] Con A responses of BB and WF splenic lymphocytes with and without added cells were measured on day 3.

[b] Counts per minute. Each value is mean of triplicate cultures ± S.D.

[c] x designates irradiated cells.

TABLE 9

II. EFFECT OF ADDITION OF IRRADIATED OR NONIRRADIATED ALLOGENEIC CELLS ON MITOGENIC RESPONSES OF BB AND WF SPLEEN CELLS

Responder (cells/well)	Counts Per Minute[a]				
	PWM Concentration (μg/ml)			PHA Concentration (%)	
	0	1.0	5.0	0.5	1.0
A = BB 0.5×10^5	1455 ± 242	7977 ± 118	6897 ± 392	6842 ± 470	4329 ± 489
B = WF 0.5×10^5	15459 ± 485	81736 ± 641	100007 ± 9988	106333 ± 1712	128479 ± 10372
A + Bx[b] 0.5×10^5 each	6511 ± 2591	7129 ± 1342	6435 ± 664	4881 ± 111	3615 ± 808
B + Ax 0.5×10^5 each	19024 ± 13958	82276 ± 9143	106082 ± 3437	88380 ± 1290	90076 ± 5484
Ax 0.5×10^5	4673 ± 3661	2819 ± 944	2475 ± 1846	1211 ± 318	1428 ± 0
Bx 0.5×10^5	1655 ± 953	1121 ± 107	1102 ± 79	533 ± 134	2046 ± 441

[a] Each value is expressed as mean of triplicate cultures ± 1 S.D.

[b] x designates irradiated cells.

TABLE 10

III. EFFECT OF ADDITION OF IRRADIATED OR NONIRRADIATED ALLOGENEIC CELLS ON MITOGENIC RESPONSES OF BB AND WF PERIPHERAL BLOOD LYMPHOCYTES

Responder (cells/well)	Counts Per Minute[a]			
	PWM Concentration (μg/ml)			Con A Concentration (μg/ml)
	0	1.0	5.0	5.0
A = BB 1.0 x 10^5	2096 ± 17	2518 ± 141	4062 ± 597	23550 ± 4566
B = WF 1.0 x 10^5	1353 ± 273	25952 ± 3302	24088 ± 3851	202255 ± 6199
A + Bx[b] 1.0 x 10^5 each	2331 ± 437	4812 ± 523	3958 ± 31	n.d.
B + Ax 1.0 x 10^5 each	1957 ± 219	n.d.	n.d.	245270 ± 1882
Ax 1.0 x 10^5	n.d.[c]	n.d.	n.d.	2037 ± 1630
Bx 1.0 x 10^5	1984 ± 444	2968 ± 661	825 ± 171	n.d.

[a] Each value is expressed as mean of triplicate cultures ± 1 S.D.

[b] x designates irradiated cells.

[c] not done

TABLE 11

MLC RESPONSES OF UNSEPARATED SPLEEN CELLS FROM BB, WF AND LEWIS RATS

Responder (cells/well)[a]	Stimulator (cells/well)[b]	Counts Per Minute[c] Expt. 1	Expt. 2	Expt. 3
A = BB (0.5)	-	3342 ± 545	515 ± 230	412 ± 94
	Bx (0.5)	2769 ± 345	1503 ± 436	n.d.
	" (1.0)	n.d.[d]	530 ± 148	389 ± 152
	" (2.0)	n.d	796 ± 220	n.d.
	Cx (1.0)	3561 ± 92	585 ± 111	1288 ± 255
	" (2.0)	3763 ± 2562	612 ± 153	n.d.
	Dx (1.0)	n.d.	n.d	305 ± 117
	Bx (0.5) + Cx (1.0)	2372 ± 150	1023 ± 25	780 ± 99
	" + " (2.0)	2475 ± 422	1837 ± 121	n.d.
	Con A sup[e]	n.d.	1481 ± 92	1551 ± 49
	" + Cx (1.0)	n.d.	n.d.	1123 ± 480
	" + " (2.0)	n.d.	1072 ± 340	n.d.
	" + Bx (1.0)	n.d.	n.d.	883 ± 250
B = WF (0.5)	-	7916 ± 1277	705 ± 66	446 ± 11
	Ax (0.5)	108441 ± 2877	6260 ± 899	3900 ± 820
	" (1.0)	n.d.	2744 ± 169	2702 ± 1047
	" (2.0)	n.d.	4656 ± 724	664 ± 125
	A (0.5)	110574 ± 1650	5473 ± 1470	2572 ± 42
	Cx (1.0)	130844 ± 404	17709 ± 2567	4679 ± 465
	" (2.0)	98728 ± 2823	15846 ± 2866	3622 ± 1142
	Dx (1.0)	n.d.	n.d.	1970 ± 710
	" (2.0)	n.d.	n.d.	879 ± 658
	Ax (0.5) + Cx (1.0)	136771 ± 3335	12005 ± 1830	3226 ± 40
	" + " (2.0)	131057 ± 13639	10333 ± 2844	n.d.

TABLE 11--extended.

	A (0.5) + Cx (1.0)	170577 ± 30519	17268 ± 1728	4675 ± 651
	" + " (2.0)	148302 ± 5404	9468 ± 430	n.d.
	D (0.5) + Cx (1.0)	n.d	n.d.	15753 ± 967
	Con A sup	n.d.	143078 ± 56	88324 ± 6880
	" + Ax (1.0)	n.d.	n.d.	85104 ± 1157
	" + Cx (1.0)	n.d.	145223 ± 4213	106705 ± 7980
	" + " (2.0)	n.d.	142652 ± 11720	n.d.
C = Lewis (0.5)	-	n.d.	1180 ± 308	6455 ± 2685
	Ax (0.5)	n.d.	n.d.	19993 ± 643
	" (1.0)	n.d.	6715 ± 142	15440 ± 2610
	" (2.0)	n.d.	10508 ± 2126	n.d.
	Bx (1.0)	n.d.	19560 ± 212	11695 ± 1350
	" (2.0)	n.d.	15939 ± 434	n.d.
	Dx (1.0)	n.d.	n.d.	17366 ± 2015
	Ax (0.5) + Bx (1.0)	n.d.	n.d.	14243 ± 538
	A (0.5) + " (1.0)	n.d.	n.d.	17594 ± 1763
	Dx (0.5) + Bx (1.0)	n.d.	n.d.	20026 ± 2659
	D (0.5) + " (1.0)	n.d.	n.d.	22160 ± 2682
D = BB[f]	-	n.d.	n.d.	804 ± 177
	Ax (1.0)	n.d.	n.d.	1253 ± 132
	Bx (1.0)	n.d.	n.d.	835 ± 185
	Cx (1.0)	n.d.	n.d.	625 ± 255
	Bx (0.5) + Cx (1.0)	n.d.	n.d.	926 ± 258

[a] All responders were used at 0.5 x 10^5 cells/well.

[b] All stimulators were used at 0.5-2.0 x 10^5 cells/well.

[c] Each value is expressed as mean of triplicate cultures ± S.D.

[d] not done

[e] 0.1 ml of MF Con A sup added to relevant wells.

[f] In experiment 3, spleens from 2 BB rats were used.

responses in vitro. Unlike WF lymphocytes, BB spleen cells were also unable to respond to Con A sup alone in the six day MLC assay. The ability of WF cells to respond to Con A sup may possibly be due to the presence of submitogenic levels of Con A that in the presence of IL 2 were sufficient for stimulation of proliferation.

No significant MLC responses were exhibited by BB spleen cells to 0.5×10^5 irradiated WF cells or to 1×10^5 irradiated cells from another BB rat. In contrast, lymphocytes from WF rats were able to proliferate to $0.5\text{-}2 \times 10^5$ irradiated BB cells in MLCs, confirming the skin grafts results. The levels of proliferation by WF lymphocytes to BB stimulators were generally lower than that seen to irradiated Lewis spleen cells. The results were variable, but no major inhibition of WF responses to Lewis stimulator cells was observed in the presence of either irradiated or nonirradiated BB spleen cells. These findings, in addition to the mitogen assays, suggest the lack of proliferative responses by BB lymphocytes in vitro was not due primarily to increased suppressor activity.

Mitogenic and MLC Responses Using Rabbit Ig Antirat Ig Purified T Cells.

To obtain highly purified populations of T lymphocytes strongly reactive in alloresponses, BB spleen cells were passed through rabbit Ig antirat Ig columns. The removal of F_c receptor-bearing spleen cells (which include many B cells, monocytes and suppressor T lymphocytes) (121)

enhanced BB proliferative responses to PHA, Con A and Con A sup in comparison to the responses seen with unseparated BB spleen cells (Tables 12 and 13). However, these augmented responses were still considerably lower than WF mitogenic responses by either unseparated or purified spleen cells. These results are further evidence that suppressor T lymphocytes are not the major cause of unresponsiveness of BB lymphocytes because removal of at least a subpopulation of suppressor T cells did not restore normal proliferative capacity to BB spleen cells when stimulated with mitogens.

Purified BB spleen cells proliferated slightly more in MLCs than unseparated cells (Tables 14-16). This was especially true when 0.5×10^5 spleen cells were used as responders per well. But again, these responses were minimal in comparison with responses by purified WF splenic lymphocytes. The levels of proliferation seen by purified BB cells to Lewis or WF stimulators were, at best, comparable to responses by WF unseparated spleen cells to BB stimulating cells. The addition of irradiated or nonirradiated unseparated BB spleen cells did not strongly inhibit WF MLC responses, but these results were variable. However, the presence of irradiated WF cells did slightly increase MLC responses by purified BB lymphocytes to Lewis stimulators, but even these improved responses were still much less than those seen with WF lymphocytes. Stimulation of BB responders was noted to Con A sup, but again, the proliferation seen was 2-fold to 4-fold less than that observed by WF

TABLE 12

I. MITOGENIC RESPONSES OF PURIFIED SPLEEN CELLS

Responder (Cells/Well)[a]		Mitogen	Counts Per Minute[c] Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
Unseparated BB	(0.5)	-	451 ± 132	711 ± 131	n.d.	1970 ± 258	973 ± 106
		1 ug/ml Con A	n.d.[e]	2095 ± 526	n.d.	24831 ± 1306	9656 ± 1928
		" + Con A sup[d]	n.d.	n.d.	n.d.	7266 ± 328	6233 ± 738
		Con A sup[d]	273 ± 197	873 ± 157	n.d.	7857 ± 92	6760 ± 285
		1% PHA	n.d.	n.d.	n.d.	3593 ± 657	1609 ± 166
Purified[b] BB	(0.5)	-	n.d.	1932 ± 272	4773 ± 259	1814 ± 181	1941 ± 216
		1 ug/ml Con A	n.d.	8284 ± 1864	n.d.	41505 ± 1040	52251 ± 749
		" + Con A sup	n.d.	n.d.	n.d.	22533 ± 1654	22678 ± 2092
		Con A sup	n.d.	39289 ± 8603	11206 ± 634	16521 ± 740	28486 ± 511
		1% PHA	n.d.	n.d.	n.d.	9507 ± 1413	7819 ± 450
Unseparated WF	(0.5)	-	515 ± 96	n.d.	n.d.	27091 ± 898	4295 ± 245
		1 ug/ml Con A	n.d.	n.d.	n.d.	362930 ± 35611	153008 ± 26
		" + Con A sup	n.d.	n.d.	n.d.	370641 ± 21075	153850 ± 7718
		Con A sup	18838 ± 2455	n.d.	n.d.	278114 ± 33833	132875 ± 7014
		1% PHA	n.d.	n.d.	n.d.	162233 ± 4713	48518 ± 1847

TABLE 12--extended.

Purified[b] WF (0.5)	–	n.d.	5545 ± 578	3931 ± 1509	5899 ± 1624	2179 ± 231
	1 ug/ml Con A	n.d.	119938 ± 5677	n.d.	146154 ± 10244	128906 ± 7243
	" + Con A sup	n.d.	n.d.	n.d.	216024 ± 8342	155216 ± 8752
	Con A sup	n.d.	198630 ± 9981	55768 ± 5014	146502 ± 18922	122799 ± 1930
	1% PHA	n.d.	n.d.	n.d.	82017 ± 222	73759 ± 1158
Unseparated Lewis (0.5)	–	539 ± 49	n.d.	n.d.	23649 ± 927	n.d.
	1 ug/ml Con A	n.d.	n.d.	n.d.	346694 ± 66	n.d.
	" + Con A sup	n.d.	n.d.	n.d.	387475 ± 3707	n.d.
	Con A sup	14387 ± 3480	n.d.	n.d.	256265 ± 4559	n.d.
	1% PHA	n.d.	n.d.	n.d.	180109 ± 712	n.d.
Purified[b] Lewis (0.5)	–	n.d.	n.d.	n.d.	1505 ± 71	n.d.
	1 ug/ml Con A	n.d.	n.d.	n.d.	38767 ± 176	n.d.
	" + Con A sup	n.d.	n.d.	n.d.	97913 ± 3225	n.d.
	Con A sup	n.d.	n.d.	n.d.	46340 ± 6814	n.d.
	1% PHA	n.d.	n.d.	n.d.	29554 ± 1595	n.d.

[a] All responders were used at 0.5×10^5 cells/well.

[b] Purified spleen cells were passed through rabbit Ig-antirat Ig columns.

[c] Each value is mean of triplicate cultures ± S.D.

[d] 0.1 ml of WF Con A sup added to each well.

[e] Not done

TABLE 13

II. MITOGENIC RESPONSES OF PURIFIED SPLEEN CELLS

Responder	(Cells/Well x 10^5)	Mitogen	Expt. 1	Expt. 2
Unseparated BB	1.0	-	676 ± 102	n.d.
		1 µg/ml Con A	9661 ± 4321	n.d.
		Con A sup[c]	n.d.[d]	n.d.
Purified[a] BB	1.0	-	1787 ± 525	4053 ± 587
		1 µg/ml Con A	39289 ± 8603	n.d.
		Con A sup	n.d.	21277 ± 1699
Purified[a] WF	1.0	-	13934 ± 433	351 ± 157
		1 µg/ml Con A	230554 ± 21032	n.d.
		Con A sup	358594 ± 9869	44952 ± 10247

[a] Purified spleen cells were passed through rabbit Ig-antirat Ig columns.

[b] Each value is expressed as mean of triplicate cultures ± S.D.

[c] 0.1 ml of WF Con A sup added to each well.

[d] Not done

TABLE 14

I. MLC RESPONSES OF PURIFIED SPLEEN CELLS FROM BB, WF AND LEWIS RATS

Responder (Cells/Well)[a]	Stimulator (Cells/Well)[b]	Counts Per Minute[c] Expt. 1	Expt. 2
A = BB (0.25)	-	676 ± 94	445 ± 72
	Bx (0.25)	1240 ± 124	1237 ± 137
	" (0.5)	986 ± 123	939 ± 196
	" (1.0)	812 ± 175	1092 ± 217
	" (2.0)	n.d.[e]	1154 ± 229
	Cx (0.25)	664 ± 105	n.d.
	" (0.5)	1423 ± 213	671 ± 165
	" (1.0)	1127 ± 237	1215 ± 235
	" (2.0)	n.d.	n.d.
	Bx (0.25) + Cx (0.25)	2907 ± 192	n.d.
	" + " (0.5)	2223 ± 626	5739 ± 816
	" + " (1.0)	2518 ± 698	1560 ± 725
	" + " (2.0)	n.d.	n.d.
	Con A sup[d]	14041 ± 11216	30897 ± 2136
	" + Cx (0.25)	21776 ± 8231	n.d.
	" + " (0.5)	29994 ± 4554	20912 ± 1920
	" + " (1.0)	26999 ± 4176	7523 ± 1913
	" + " (2.0)	n.d.	n.d.
B = WF (0.25)	-	7218 ± 3428	3291 ± 689
	Ax (0.25)	12942 ± 367	4162 ± 200
	" (0.5)	18323 ± 596	10138 ± 3839
	" (1.0)	5923 ± 1237	16478 ± 1207
	" (2.0)	n.d.	n.d.
	A (0.25)	9676 ± 82	7497 ± 1959

TABLE 14--extended.

	Cx (0.25)	24407 ± 3655	n.d.
	" (0.5)	31223 ± 4232	51176 ± 934
	" (1.0)	43031 ± 20594	70704 ± 1492
	" (2.0)	n.d.	84221 ± 3507
	Ax (0.25) + Cx (0.25)	14591 ± 7080	n.d.
	" + " (0.5)	27871 ± 4755	53414 ± 3772
	" + " (1.0)	49403 ± 8278	103099 ± 3443
	" + " (2.0)	n.d.	88840 ± 5132
	A (0.25) + Cx (0.25)	14897 ± 4981	n.d.
	" + " (0.5)	30928 ± 4272	59194 ± 10402
	" + " (1.0)	39400 ± 5883	85855 ± 7407
	" + " (2.0)	n.d.	99303 ± 5118
	Con A sup	63883 ± 10038	58015 ± 6453
	" + Cx (0.25)	60878 ± 613	n.d.
	" + " (0.5)	44642 ± 3533	103680 ± 3360
	" + " (1.0)	51319 ± 1684	106304 ± 1574
	" + " (2.0)	n.d.	149534 ± 2526
C = Lewis (0.25)	-	16769 ± 3389	2912 ± 690
	Ax (0.5)	28681 ± 1282	26483 ± 4564
	" (1.0)	n.d.	31363 ± 7818
	Bx (0.5)	19708 ± 3614	24212 ± 3213
	" (1.0)	n.d.	7080 ± 1012
	" (2.0)	n.d.	15153 ± 592

[a] Rabbit Ig antirat Ig treated spleen cells were used as responders at 0.25 x 10^5 cells/well.

[b] Unseparated spleen cells were used as stimulators at 0.25-2.0 x 10^5 cells/well.

[c] Each value is expressed as mean of triplicate cultures ± S.D.

[d] 0.1 ml of WF Con A sup was added to each well.

[e] Not done

TABLE 15

II. MLC RESPONSES OF PURIFIED SPLEEN CELLS FROM BB, WF AND LEWIS RATS

Responder (Cells/Well)[a]	Stimulator (Cells/Well)[b]	Counts Per Minute[c]			
		Expt. 1	Expt. 2	Expt. 3	Expt. 4
A = BB (0.5)	-	2233 ± 1130	715 ± 21	961 ± 147	2545 ± 245
	Bx (0.5)	2135 ± 632	1239 ± 188	1485 ± 536	1721 ± 137
	" (1.0)	2329 ± 474	1269 ± 714	2053 ± 32	2139 ± 288
	" (2.0)	1415 ± 444	1218 ± 978	5256 ± 953	2011 ± 450
	Cx (0.5)	2484 ± 68	2318 ± 118	1872 ± 991	n.d.
	" (1.0)	2662 ± 223	2352 ± 254	2732 ± 362	1521 ± 513
	" (2.0)	2766 ± 28	4314 ± 922	5651 ± 301	2083 ± 332
	Bx (0.5) + Cx (0.5)	3972 ± 48	7077 ± 291	11590 ± 1036	n.d.
	" + " (1.0)	5533 ± 449	4409 ± 1652	1918 ± 1095	11348 ± 2095
	" + " (2.0)	1802 ± 14	4329 ± 853	1576 ± 560	15658 ± 6228
	Cxn A sup[d]	57258 ± 9327	52189 ± 480	45222 ± 8189	59534 ± 7818
	" + Cx (0.5)	34192 ± 1611	51503 ± 1269	n.d.[e]	n.d.
	" + " (1.0)	21884 ± 3999	25489 ± 1538	n.d.	66912 ± 11072
	" + " (2.0)	8738 ± 2337	9332 ± 91	n.d.	50283 ± 9293
B = WF (0.5)	-	12993 ± 986	9610 ± 1830	4287 ± 1647	29968 ± 1971
	Ax (0.5)	25070 ± 1726	40613 ± 9684	22175 ± 2046	54508 ± 7353
	" (1.0)	31849 ± 1616	26650 ± 8081	49987 ± 6894	37502 ± 1167
	" (2.0)	64484 ± 6997	19810 ± 1277	28011 ± 1026	40601 ± 8772
	A (0.5)	53077 ± 5663	45757 ± 6213	18319 ± 6409	73014 ± 3559
	Cx (0.5)	43811 ± 5665	86202 ± 1088	41363 ± 4823	n.d.
	" (1.0)	85191 ± 1762	107759 ± 2685	40619 ± 6793	181268 ± 11894
	" (2.0)	116212 ± 5950	117505 ± 7199	59841 ± 9125	84645 ± 6379

TABLE 15—extended.

	Ax (0.5) + Cx (0.5)	50742 ± 2236	52419 ± 5038	35833 ± 3231	n.d.
	" + " (1.0)	56669 ± 14589	93079 ± 4340	36087 ± 8130	161905 ± 10950
	" + " (2.0)	91666 ± 1816	126262 ± 14651	60852 ± 2886	65956 ± 3371
	A (0.5) + Cx (0.5)	76911 ± 3207	62198 ± 66	41008 ± 1229	n.d.
	" + " (1.0)	84814 ± 2497	94954 ± 7347	45451 ± 1078	125788 ± 8754
	" + " (2.0)	117983 ± 6885	110192 ± 16236	54014 ± 1864	105592 ± 7678
	Cm A sup	123625 ± 1669	107632 ± 6091	84132 ± 4909	151792 ± 20574
	" + Cx (0.5)	n.d.	112544 ± 5182	n.d.	n.d.
	" + " (1.0)	n.d.	103623 ± 3416	n.d.	132912 ± 1609
	" + " (2.0)	n.d.	92436 ± 4320	n.d.	123526 ± 1314
C = Lewis (0.5)	–	15784 ± 1009	12177 ± 1289	n.d.	21663 ± 7698
	Ax (0.5)	29530 ± 4681	25309 ± 2510	n.d.	n.d.
	" (1.0)	50343 ± 10262	37655 ± 8828	n.d.	59077 ± 4865
	" (2.0)	91072 ± 7413	n.d.	n.d.	82321 ± 1981
	Bx (0.5)	35452 ± 6126	17128 ± 1230	n.d.	n.d.
	" (1.0)	45351 ± 3223	37830 ± 3955	n.d.	51747 ± 6677
	" (2.0)	54859 ± 1768	68045 ± 10402	n.d.	55670 ± 20746
	Cm A sup	n.d.	n.d.	n.d.	112583 ± 24165

[a] Rabbit Ig antirat Ig treated spleen cells were used as all responders at 0.5×10^5 cells/well.

[b] Unseparated spleen cells were used as stimulators at $0.5\text{-}2.0 \times 10^5$ cells/well.

[c] Each value is expressed as mean of triplicate cultures ± S.D.

[d] 0.1 ml of MF Cm A sup was added to each well.

[e] Not done

TABLE 16

III. MLC RESPONSES OF PURIFIED SPLEEN CELLS FROM BB, WF AND LEWIS RATS

Responder (Cells/Well)[a]	Stimulator (Cells/Well)[b]	Counts Per Minute[c] Expt. 1	Expt. 2
A = BB (1.0)	-	2199 ± 250	4797 ± 1016
	Bx (1.0)	2103 ± 801	3226 ± 571
	" (2.0)	2728 ± 172	3329 ± 254
	Cx (1.0)	5346 ± 1876	3679 ± 64
	" (2.0)	2844 ± 62	3164 ± 62
	Con A sup[d]	n.d.[e]	87825 ± 2619
	" + Cx (1.0)	n.d.	29633 ± 5397
	" + " (2.0)	n.d.	12742 ± 1597
B = WF (1.0)	-	74155 ± 8028	39977 ± 17259
	Ax (1.0)	128158 ± 558	71425 ± 12699
	" (2.0)	101730 ± 7025	74309 ± 5049
	Cx (1.0)	104270 ± 2589	69389 ± 11321
	" (2.0)	178659 ± 175	119614 ± 17349

TABLE 16--extended.

	Con A sup	168991 ± 5435	125904 ± 962
	" + Cx (1.0)	n.d.	n.d.
	" + " (2.0)	n.d.	n.d.
C = Lewis (1.0)	-	n.d.	35052 ± 6513
	Ax (1.0)	n.d.	55566 ± 3239
	" (2.0)	n.d.	98563 ± 18159
	Bx (1.0)	n.d.	42363 ± 10671
	" (2.0)	n.d.	45317 ± 10961

[a] Rabbit Ig antirat Ig treated spleen cells were used as responders at 1.0×10^5 cells/well.

[b] Unseparated spleen cells were used as stimultors at $1.0\text{-}2.0 \times 10^5$ cells/well.

[c] Each value is expressed as mean of triplicate cultures ± S.D.

[d] 0.1 ml of WF Con A sup added to each well.

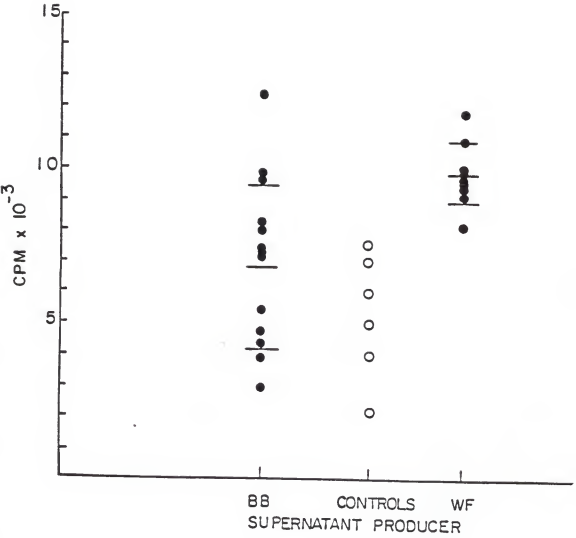
[e] Not done

spleen cells to Con A sup. MLC responses by BB lymphocytes to Lewis stimulators in the presence of Con A sup were only comparable to WF MLC results without Con A sup. The proliferation was most likely due to the Con A sup alone as the counts were not generally greater when Lewis stimulators were added. Thus, the addition of Con A sup did not trigger BB lymphoctes to specifically respond to allogeneic Lewis cells.

Ability of Spleen Cells to Produce Lymphokines (IL 2). As a possible cause of immunoincompetence, the ability of BB spleen cells to produce IL 2 was determined. Supernatants obtained from the Con A activation of BB spleen cells contained significant amounts of IL 2 as measured by the proliferation of IL 2-dependent cytotoxic T cells (Figure 28). However, the levels of IL 2 in BB Con A sups were extremely variable, while all WF Con A sups had similar amounts of IL 2. BB lymphocytes were thus able to produce IL 2 but were probably deficient in their ability to respond to this factor as indicated by the previous studies.

Ability of Thymocytes to Respond to Mitogens. In order to determine if the T lymphocyte defect was only peripheral or also included the thymus, mitogenic responses of BB and WF thymocytes were compared. In sharp contrast to results obtained with spleen cells and PBL, thymocytes from BB rats proliferated as well to both Con A and Con A sup as did WF

FIGURE 28. Proliferative activities (cpm) of Con A supernatants from 13 diabetic and nondiabetic BB rats and 9 WF rats on a murine IL 2-dependent cytotoxic T cell line. Activities of 6 dilutions of a control murine Con A supernatant are also indicated. Plotted in increasing order, the dilutions used: 1.5%, 3.1%, 6.3%, 12.5%, 25% and 50%. Lines indicating mean $\pm$ 1 S.D. of IL 2 activity of Con A sups from each group are shown.


15
10
5
CPM x 10^{-3}
BB
CONTROLS
WF
SUPERNATANT PRODUCER

thymocytes (Table 17). The variability in levels of proliferation between the three experiments was due to differences in ages of the thymuses used, however, the BB and WF thymuses were age matched for each experiment. The ages of the thymuses used were 15 days, 26 days and 21 days for experiments 1, 2 and 3 respectively. From these findings, the defect in T lymphocyte immunocompetence would seem to be expressed only in peripheral T cells.

Lymphoid Tissue Histology. Because the above findings suggested that thymocytes from BB rats were functionally normal, while splenic lymphocytes and PBL were obviously immunoincompetent, histological examinations of BB thymuses, spleens and lymph nodes were performed for comparison with WF lymphoid tissues. No significant differences were seen at the light microscopic level between thymuses from BB rats (Figure 29) and WF rats (Figure 30). In sharp contrast, T cell but not B cell-dependent areas of spleens (Figure 31) and lymph nodes (Figure 33) from BB rats were severely depleted of lymphocytes in comparison with spleens (Figure 32) and lymph nodes (Figure 34) from WF rats.

Gamma Globulin Levels. Serum gamma globulin levels were measured in BB rats as a gross indication of B lymphocyte function. The levels of immunoglobulins in sera from BB rats with and without IDD were not significantly different from those measured in WF controls (Table 18). These

TABLE 17

MITOGENIC RESPONSES OF BB AND WF THYMOCYTES

Responder	(Cells/Well)	Mitogen Added	Counts Per Minute[a] Expt. 1	Expt. 2	Expt. 3
BB	0.5×10^5	-	n.d.[c]	4724 ± 1275	1075 ± 363
		0.1 µg/ml Con A	n.d.	n.d.	1300 ± 250
		0.1 µg/ml Con A + sup[b]	n.d.	n.d.	52296 ± 6632
		1.0 µg/ml Con A	n.d.	112208 ± 1000	12353 ± 2753
		1.0 µg/ml Con A + sup	n.d.	320578 ± 3440	67367 ± 2886
		sup	n.d.	n.d.	53851 ± 6993
	1.0×10^5	-	950 ± 6	455 ± 313	2153 ± 287
		0.1 µg/ml Con A	n.d.	n.d.	1203 ± 212
		0.1 µg/ml Con A + sup	n.d.	n.d.	91726 ± 8295
		1.0 µg/ml Con A	3812 ± 37	176397 ± 49111	28292 ± 1001
		1.0 µg/ml Con A + sup	23225 ± 2685	323411 ± 7001	133644 ± 18797
		sup	32606 ± 689	n.d.	117650 ± 1716

TABLE 17--extended.

WF	0.5×10^5	-	n.d.	5806 ± 2465	1887 ± 592
		0.1 μg/ml Con A	n.d.	n.d.	5303 ± 1170
		0.1 μg/ml Con A + sup	n.d.	n.d.	96032 ± 6500
		1.0 μg/ml Con A	n.d.	156783 ± 11178	37747 ± 2108
		1.0 μg/ml Con A + sup	n.d.	327965 ± 14647	136274 ± 1854
		sup	n.d.	n.d.	109054 ± 5122
	1.0×10^5	-	1707 ± 453	7287 ± 1054	8080 ± 1629
		0.1 μg/ml Con A	n.d.	n.d.	5393 ± 813
		0.1 μg/ml Con A + sup	n.d.	n.d.	150478 ± 14030
		1.0 μg/ml Con A	3892 ± 518	205707 ± 7302	60307 ± 6737
		1.0 μg/ml Con A + sup	27281 ± 6875	318668 ± 8665	179770 ± 3557
		sup	12235 ± 506	n.d.	180830 ± 642

[a] Con A responses of thymocytes were measured on day 3. Each value is mean of triplicate cultures ± S.D.

[b] 0.1 ml of WF Con A sup was added to each well.

[c] Not done

FIGURE 29. Hematoxylin and eosin stained section of a thymus from a nondiabetic BB rat at 21 days of age.

FIGURE 30. Hematoxylin and eosin stained section of a thymus from a WF rat at 21 days of age.

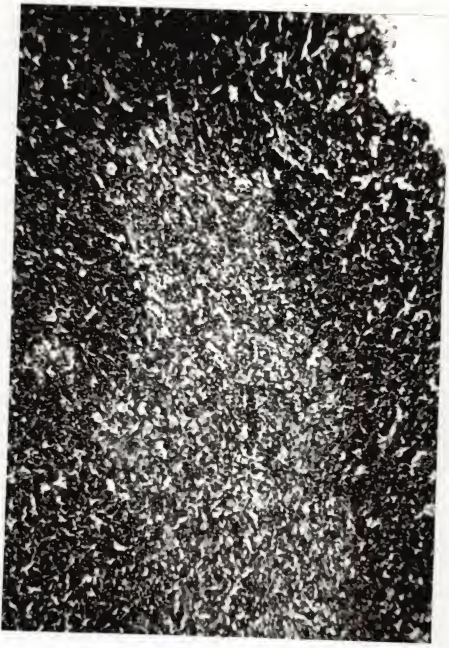


FIGURE 31. Section of spleen from a nondiabetic BB rat stained with hematoxylin and eosin. Normal B cell-dependent areas (black arrow) are present, but a virtual absence of T cell-dependent areas around arterioles (clear arrow) are seen.

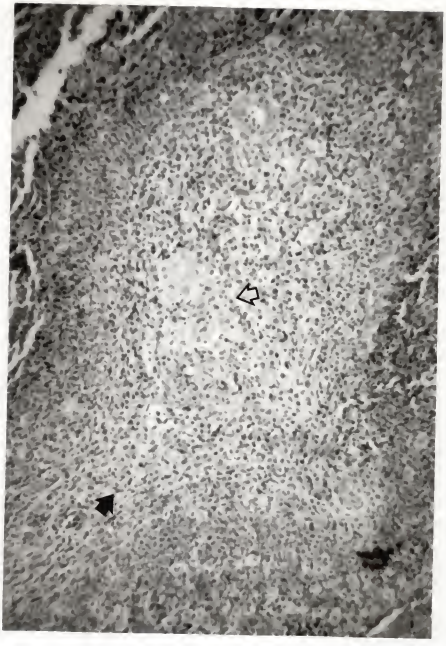


FIGURE 32. Hematoxylin and eosin stained section of spleen from a WF rat. Both B cell areas (black arrow) and T cell-dependent areas (clear arrow) around arterioles are present.

FIGURE 33. Section of lymph node from a nondiabetic BB rat stained with hematoxylin and eosin. B cell follicles (black arrow) are relatively normal, but parafollicular area is severely depleted of T lymphocytes (clear arrow).

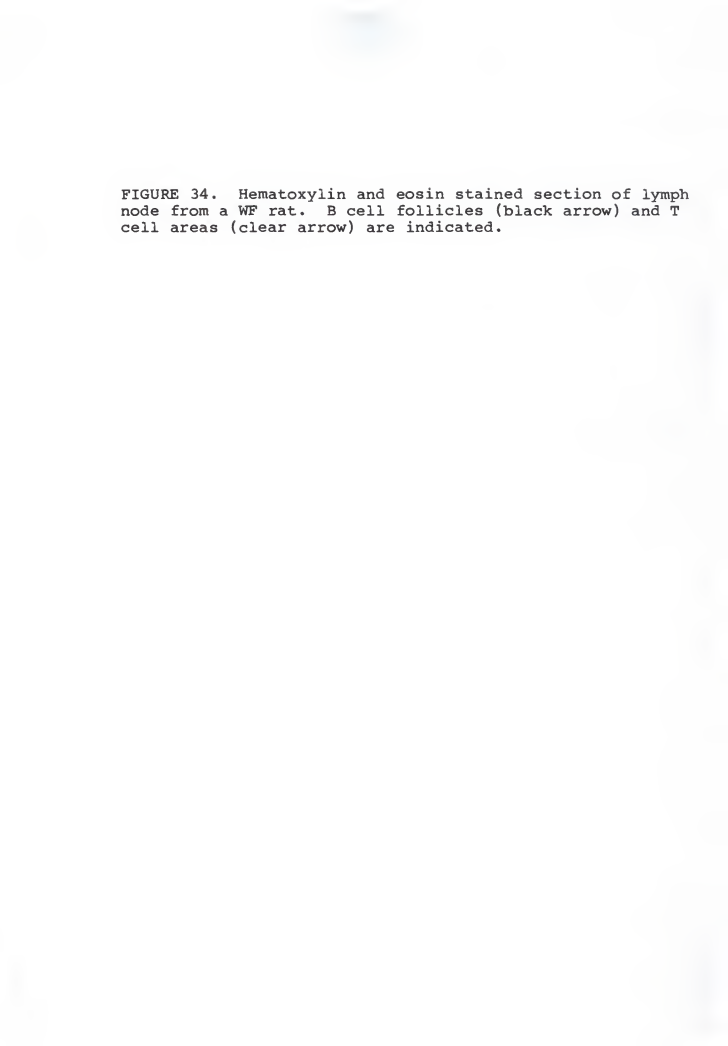


FIGURE 34. Hematoxylin and eosin stained section of lymph node from a WF rat. B cell follicles (black arrow) and T cell areas (clear arrow) are indicated.

TABLE 18

SERUM GAMMA GLOBULIN LEVELS IN BB AND WF RATS

Sera	% Gamma Globulins in Serum[a]
10 BB rats with IDD	5.4 ± 1.7
7 BB rats without IDD	4.4 ± 2.8
7 WF rats	5.2 ± 1.5

[a] Each value is mean ± S.D.

results suggested that the functions as well as the numbers of B lymphocytes may possibly be unimpaired in these BB rats.

Transfer Experiments. Attempts were made to transfer IDD with pancreas extracts and cells from the spleen, the mesenteric lymph nodes, and the peritoneal cavity of diabetic BB rats to nondiabetic BB, WF and BB x WF F1 hybrid animals. These studies were performed because positive results would provide definitive proof for an autoimmune etiology for IDD in the BB rat.

As shown in Table 19, no combination of cell types, cell numbers or recipients resulted in the development of hyperglycemia, as defined by blood glucose levels greater than 250 mg/dl, or pancreatic insulitis in any rats. PCA were also not detected in the sera of any recipients, although they were sought in the sera for more than 90 days. SMA were demonstrated in the sera of four WF recipients, but as shown previously in Table 1, SMA were normally found in some WF rats.

Genetics of IDD. Studies of the mode of inheritance of IDD and PCA were carried out in order to see genetic similarities between the BB rat and humans with IDD. In addition to the findings that BB x WF F1 rats do not develop IDD (Table 1), further indications that IDD in the BB rat probably does not have a dominant inheritance include

TABLE 19

SUMMARY OF TRANSFER EXPERIMENTS

Expt.	BB Rat Donor	Duration of IDD	Recipient	Transferred Cells[a] No. of Rats	Cell Source	Number of Cells Transferred to Each	Number with Hyperglycemia[b]	Number with Insulitis[c]	Number with Autoantibodies[d]
1	4/1	Onset	8 WF	3	Spleen	10.5×10^6	0[f]	0	1[g]
				2	Lymph nodes	5×10^6	0	0	0
				2	Peritoneal cavity	4×10^6	0	0	0
				1	No cells		0	0	0
2	2/1	Onset	8 WF	3	Spleen	33×10^6	0	0	1
				2	Lymph nodes	3×10^6	0	0	0
				2	Peritoneal cavity	3×10^6	0	0	1
				1	No cells		0	0	0
3	1/2	Onset	8 WFx[e]	3	Spleen	13.5×10^6	0	0	0
				2	Lymph nodes	5×10^6	0	0	0
				2	Peritoneal cavity	3×10^6	0	0	1
				1	No cells		0	0	0
4	7/11	9 Days	1 BB x WF F1x	1	Pancreas	-	0	0	0

TABLE 19--extended.

5	9/10	3 Days	3 BB x WF F1x	2	Spleen + Lymph nodes 70 x 10^6	0	0	0
				1	Spleen + Lymph nodes 70 x 10^6 + Pancreas	0	0	0
6	7/8	7 Days	3 BB x WF F1x	1	Spleen + Lymph nodes 60 x 10^6	0	0	0
				1	Spleen + Lymph nodes 60 x 10^6 + Pancreas	0	0	0
			1 BB w/o IDD	1	Spleen + Lymph nodes 60 x 10^6	0	0	0

[a] Cells from spleen, mesenteric lymph nodes and peritoneal cavity were injected into tail veins of recipients. Pancreas suspension was given intraperitoneally.

[b] Hyperglycemia defined as blood glucose levels greater than 250 mg/dl.

[c] Pancreases were removed from recipients 90-120 days after receiving donor cells.

[d] ICA, TMA, PCA and SMA were sought in recipient rats.

[e] x designates recipients irradiated with 300-350 rads 24 hours before transfer.

[f] Blood glucose levels were run on recipients on day 0, day 3 and every 7 days after for at least 90 days.

[g] ICA, TMA or PCA were never detected in any BB rats. Four rats positive for autoantibodies had SMA.

observations that matings between diabetic BB rats resulted in litters with frequencies of IDD of only 50%, while matings between 2 nondiabetic BB rats produced a few diabetic offspring (Table 20). Interestingly, matings of nondiabetic BB rats with normal glucose tolerance tests who were both PCA-positive, produced offspring with IDD. Crosses of diabetic BB rats without PCA resulted in offspring positive for PCA. These results raise questions as to whether IDD and PCA are due to the same or separate genes.

As was the case with BB x WF F1 rats, BB x Lewis F1 animals did not develop either IDD or PCA (Table 21). None of the rats in F2 litters from matings of either BB x WF F1 rats or BB x Lewis F1 animals were positive for either IDD or PCA. However, many of these F2 rats, especially from the Lewis crosses, were not yet old enough at the time of this writing to develop these autoimmunities.

TABLE 20

FREQUENCIES OF IDD AND PCA IN SOME MATING COMBINATIONS OF BB RATS

BB Matings[a]		Offspring			
Male	Female	Total Litters	Total Rats	IDD^+ (no.)	PCA^+ (no.)
$IDD^+ PCA^{+/-}$[b]	$IDD^+ PCA^{+/-}$	8	20	10	6
$IDD^+ PCA^{+/-}$	$IDD^- PCA^{+/-}$	9	42	15	14
$IDD^- PCA^{+/-}$	$IDD^- PCA^{+/-}$	3	22	3	2[c]
$IDD^{+/-}$[d] PCA^+	$IDD^{+/-} PCA^+$	8	36	8	5
$IDD^{+/-} PCA^+$	$IDD^{+/-} PCA^-$	7	21	9	7
$IDD^{+/-} PCA^-$	$IDD^{+/-} PCA^+$	2	6	3	4
$IDD^{+/-} PCA^-$	$IDD^{+/-} PCA^-$	3	21	8	6
$IDD^+ PCA^+$	$IDD^+ PCA^+$	1	2	1	0
$IDD^+ PCA^-$	$IDD^+ PCA^-$	1	8	3	4
$IDD^- PCA^-$	$IDD^- PCA^-$	-	-	-	-
$IDD^- PCA^+$	$IDD^- PCA^+$	3	22	3	2[c]

TABLE 20--extended.

IDD^+ PCA^+	IDD^- PCA^+	4	12	4	3
IDD^+ PCA^+	IDD^+ PCA^-	5	9	7	2
IDD^+ PCA^-	IDD^- PCA^+	1	5	3	4
IDD^+ PCA^+	IDD^- PCA^-	2	12	2	5
IDD^+ PCA^-	IDD^- PCA^-	2	13	5	2
IDD^+ PCA^-	IDD^+ PCA^+	1	1	0	0

[a] Only litters born before 1/1/82 are included because the final frequencies of IDD and PCA in younger litters have not yet been reached.

[b] The frequency of IDD was calculated independently of PCA (without regards to the presence or absence of PCA).

[c] The frequency of PCA is only for 1 litter of 7 rats, as PCA were not sought in the other 2 litters.

[d] The frequency of PCA was calculated independently of IDD.

TABLE 21

RESULTS OF MATINGS OF MALE BB DIABETIC RATS WITH BOTH FEMALE WF AND LEWIS RATS

Matings		Offspring			
Male BB	Female	Total Litters	Total Rats	IDD^+ (no.).	PCA^+ (no.)
4/2 IDD^+ PCA^-	WF	1	11	0	0
1/1 IDD^+ PCA^+	WF	1	10	0	0
5/2 IDD^+ PCA^+	WF	1	9	0	0
12/3 IDD^+ PCA^-	WF	1	12	0	0
21/2 IDD^+ PCA^+	WF	1	8	0	0
7/1 IDD^+ PCA^+	Lewis	2	14	0	0
1/1 x WF	1/1 x WF	1	3	0	0
1/1 x WF	4/2 x WF	1	1	0	0
5/2 x WF	5/2 x WF	2	0	0	0
12/3 x WF	12/3 x WF	1	4	0[a]	0[a]
7/1 x Lewis	7/1 x Lewis	9	57	0[a]	0[a]

[a] Not all the F2 rats were yet past the age period for susceptibility to IDD and PCA.

DISCUSSION

The BB Rat As A Model Of IDD And Organ-Specific Autoimmunity

There are a number of similarities between the IDD observed in humans and in BB rats. The age period that BB rats were most susceptible to IDD occurred during late puberty and corresponds to the second peak of incidence of human IDD, which is during adolescence (122). In both man and rat, this time of onset of IDD coincides with an accelerated growth period during which insulin requirements increase.

Several important findings suggest that the pancreatic beta cell destruction observed in human IDD may be of immunological origin. The degree of similarity of immunological characteristics between diabetic BB rats and human patients with IDD was therefore of great interest and an important stimulus for these studies. Initial findings have confirmed that pancreases from all BB rats with IDD in this laboratory had lymphocytic infiltrations of the islets of Langerhans as has been previously reported in other BB rats and in man.

Studies of multiplex families with IDD have confirmed that the disease is clearly inherited with HLA haplotypes, while population studies have demonstrated that IDD is

almost universally associated with HLA DR3 and/or HLA DR4-bearing haplotypes. The mode of transmission of IDD in man is currently thought to be complex; however, with the probability of polygenic inheritance as modified by environmental factors. In observations reported here, no BB x WF F1 rats or BB x Lewis F1 animals developed IDD, indicating that IDD in the BB rat, as is the case with IDD in Caucasian populations, is not dominantly inherited as a single gene. The incidences of IDD in the offspring of matings between two diabetic BB rats or two nondiabetic BB animals could be explained by inheritance as a single autosomal recessive gene with low penetrance or as multiple genes. Only the F2 animals produced by crosses of BB x WF F1 rats or BB x Lewis F1 rats can provide definitive evidence for a recessive mode of inheritance for IDD in the BB rat. At the time of this writing, most F2 animals in this laboratory were not old enough to develop IDD. However, Colle et al. (123) has recently found that a few BB x Lewis F2 rats developed IDD, while other rats only developed pancreatic insulitis, suggesting that IDD may result from the interaction of at least two genes. The F2 rats that developed IDD all had at least one $RT.1^{u}$ (BB) bearing haplotype. Because only four F2 rats have actually developed IDD in Colle's findings to date, more extensive results are needed before the mode of inheritance of IDD in the BB rat is actually proven. Notwithstanding, the studies to date support a polygenic inheritance involving major histocompatibility complex genes and

variable penetrance in both BB rats with IDD and in human insulin-dependent diabetics.

The identification of ICA provided major support for an autoimmune etiology for human IDD. In contrast to human patients with IDD, ICA were not found in the sera of any BB rats at anytime before, during or after onset of the disease. These autoantibodies were also not detected on pancreas sections from BB rats at the onset of IDD, indicating that antibodies against IDD-specific cytoplasmic antigens were not in fact produced in these animals. However, these studies have not excluded the presence of autoantibodies reactive with pancreatic beta cell surface antigens (ICSA) in BB rats, which indeed have recently been reported (101). It is not yet known whether ICSA were complement fixing or cytotoxic in BB rats. ICA were not demonstrated by indirect immunofluorescence using antirat total immunoglobulins in addition to antirat IgG as second antibodies, suggesting that ICA of the IgM class were also not produced by these rats. Similarly, ICA of the IgM class have not been identified in human patients with IDD (124). Thus, no readily apparent marker for the disease process resulting in IDD is available in the BB rat to identify susceptible prediabetic animals, although detection of ICSA, albeit cumbersome to detect, has this potential.

In contrast to reports in human IDD (15), neither thyroid microsomal nor adrenocortical autoantibodies were detected in any BB rats. The absence of ICA, thyroid

microsomal antibodies, and adrenal antibodies in diabetic BB rats is in striking contrast to human IDD, and might support the hypothesis that IDD in the BB rat may not be the same disease or have the same pathogenesis as human IDD.

However, in similarity with human IDD, PCA were detected in a considerable number of BB rats, although no BB rats with PCA had functional abnormalities of the gastric parietal cells such as achlorhydria. Human PCA-positive diabetic patients with achlorhydria are known to absorb protein-bound vitamin B12 poorly and be susceptible to vitamin B12 deficiences (125). However, in comparison to the serum vitamin B12 levels measured in BB rats without PCA and in control WF rats, serum vitamin B12 levels were not significantly lower in BB rats with PCA, probably because the animals had normal amounts of hydrochloric acid and sufficient amounts of intrinsic factor. Achlorhydria has also been shown to decrease the absorption of iron (126). No PCA-positive BB rats had significantly lower serum iron levels than BB rats without detectable PCA or control WF animals.

Notwithstanding, the presence of PCA was associated with histological evidence of chronic lymphocytic gastritis, squamous metaplasia, and degrees of loss of the normal gastric mucosal cells. It remains possible that BB rats may develop atrophic gastritis, which is seen in some human patients with PCA (127), at a greater age than the animals in this study. These rats were followed for nine months,

but this period of observation might have been insufficient to develop more striking gastric lesions and/or serum vitamin B12 and iron deficiency states.

No sex difference was noted in the frequencies of PCA in either BB/O or BB/W rats, which is in contrast to PCA in human IDD where females are more commonly affected. PCA first appeared and rapidly increased in frequency during the age span that the BB rats were most susceptible to the development of IDD and did not further increase with age after this critical period. Thus, it appears that BB rats with IDD and gastric autoimmunity have onsets of these disease processes at the same time of life. However, the rates of progression of these individual diseases to clinical states obviously vary, as no clinical disease state of the gastric parietal cells occurred. In studies by Riley and Maclaren of human IDD, thyroid microsomal autoantibodies and PCA were shown in the majority of cases to be present at the time of onset of IDD (127). Thus, these findings in the BB rat are of great interest, since they suggest a similarity between BB rats and human insulin-dependent diabetic patients, and indicate an underlying defect in tolerance occurring at an early age in both humans and BB rats with IDD.

As was the case with IDD, no WF, BB x WF F1, or BB x Lewis F1 rats were positive for PCA, suggesting that PCA were not the result of a single dominant gene. Assuming that PCA represents some genetic predisposition, it is of

interest to assess whether this predisposition is the same one that leads to destruction of the beta cells of the pancreas and IDD. Two findings suggest that the genes for PCA and IDD might be separate. The frequencies of PCA in BB/O and BB/W rats was different (although not significantly so) despite similar rates of IDD. Furthermore, some rats without discernible IDD including normal glucose tolerance tests were found to have PCA. However, observations of matings between diabetic BB rats without PCA producing litters with PCA-positive rats, while nondiabetic BB rats with PCA produced offspring with IDD, do suggest that the gene for IDD and PCA may be one and the same, possibly with reduced penetrance. Alternatively, as hypothesized above, the genes for IDD and PCA may be separate but interact to increase the degree of penetrance of the other. Further proof of these contentions should be provided by the F2 litters obtained from matings of male diabetic PCA-positive BB rats with female Lewis or WF animals which have not yet reached the age for susceptibility to IDD and PCA.

Other autoantibodies were sought and found in BB rats. SMA occurred in high frequencies, but these autoantibodies were not associated with either PCA or IDD. That a low frequency of SMA was found in BB x WF F1 animals and WF control rats who never developed IDD, suggests that the process leading to formation of SMA is independent of the processes resulting in IDD and PCA. Thus, as is the case with autoimmune endocrinopathies in man, the organ-specific

autoimmunities do not seem to be genetically associated with systemic autoimmunity like SMA in BB rats. Because thyroid colloid antibodies were found in only a few BB rats, these observations are insufficient to relate the presence of thyroid colloid antibodies to the other autoimmune processes resulting in IDD and PCA.

In summary, in respect to frequencies of autoantibodies and associated diseases, there are both similarities and differences between BB rats and human patients with IDD. The number of autoantibodies present in BB rats suggests that the strain has an underlying autoimmune diathesis, of which IDD may be only one possible result. The BB rat appears to have a general genetic predisposition for autoimmunity, with IDD and PCA being the result of either the same or separate genetic influences and SMA occurring independently of either PCA or IDD.

Although the presence of autoantibodies provides further evidence that the IDD seen in the BB rat may result from an autoimmune process, transfers of pancreas suspensions, lymphocytes and peritoneal macrophages from diabetic BB rats did not result in the development of IDD or pancreatic insulitis in any recipient animals. However, serum was not transferred from BB rats with IDD neither alone or in combination with lymphocytes and pancreas, which may have contributed to the negative results obtained if specific humoral immunity or antibody-dependent cell-mediated cytotoxicity were involved. Furthermore,

there may have been an unknown requirement for the target pancreatic beta cells to express IDD-specific or BB strain-specific antigens. This important area is one in which further studies are obviously required.

The BB Rat As A Model Of Immunodeficiency

Due to observations of the increased susceptibility of BB rats to opportunistic infections especially of the respiratory tract, the status of immunocompetence in the BB rat was next studied. BB rats of all ages exhibited a profound lymphopenia which was independent of the presence of IDD. This lymphopenia was due to the specific loss of T cells as the absolute numbers of B lymphocytes observed in BB rats were comparable to the numbers observed in WF controls. PMNs were increased in BB rats, most likely as a response to the various chronic respiratory infections normally present in these animals. Monocytes and eosinophils were found in similar proportions in both BB and WF animals, albeit some BB rats had an obvious eosinophila. As a possible explanation for the severely decreased numbers of peripheral T cells, thymocytotoxic autoantibodies were sought and found in sera from many BB rats with and without IDD. The actual role, if any, of these antibodies in the development of lymphopenia in the BB rat remains to be explored, however, their contribution seems likely.

W3/25-positive cells are thought to be the proliferating helper cells in MLCs, the T helper cell in the

production of antihapten antibodies, and the initiating cell in graft versus host reactions (128). MRC OX8-positive cells are considered to be responsible for suppression of allograft rejection (128). The T lymphopenia in BB rats involved both major T cell subpopulations, however, W3/25-positive cells were affected more severely than MRC OX8-positive cells. An inversion of the ratio of the W3/25-positive subset to the MRC OX8-positive subset to less than one occurred in all BB rats with increasing age and was probably a reflection of the specific decrease in circulating helper T cells. Normally, as observed in younger BB rats and WF controls, the numbers of helper T lymphocytes were greater than the numbers of cytotoxic/suppressor T cells. Interestingly, this ratio inversion occurred during the same age period (90 to 120 days of age) that the BB rats were most susceptible to the development of IDD and PCA. This finding suggests that the abnormality in immunological regulation acquired by BB animals with relatively increased cytotoxic/suppressor T cells may partly contribute to or be the result of the development of IDD and PCA. However, even BB rats who did not develop overt autoimmunity had decreased numbers of helper T cells and cytotoxic/suppressor T lymphocytes and inverted W3/25-positive subset to MRC OX8-positive subset ratios.

Concurrently with the severe depressions of both major T cell subsets and the abnormal susceptibility of these animals to opportunistic infections, severe defects in both

in vivo and in vitro T cell-mediated immune responses were noted in all BB rats studied, irrespective of age or the presence of IDD. Defective graft rejection across both major and minor histocompatibility barriers was observed in these animals. The inability of BB rats to reject allografts normally probably was related to the severe T lymphopenia present in these animals.

Con A, PHA and PWM are considered to be mainly T lymphocyte mitogens in the rat, but B cells may also be stimulated to divide (112,114,129). Even though these mitogens are extremely potent nonspecific stimulators of T cell proliferation, lymphocytes from BB rats were unable to mount proliferative responses comparable to those by lymphocytes from WF rats to any concentration of PWM, PHA, Con A or Con A sup. The lack of mitogenic responses by BB spleen cells or PBL probably reflects to a large degree the fewer T lymphocytes present in BB spleen cells or PBL compared to the number of responding T cells in the same concentrations of WF spleen cells or PBL. Comparable mitogenic responses by BB and WF lymphocytes were only observed when at least ten times fewer WF spleen cells or PBL than BB cells were used. However, the absolute numbers of peripheral blood T lymphocytes in BB rats were only two-fold to three-fold less than the absolute numbers of T cells seen in WF animals, suggesting that decreased numbers of T lymphocytes were not the only factor contributing to the lack of mitogenic responses by BB rats in vitro.

The MLC is considered to be an in vitro correlate of the process which occurs in vivo during allograft rejection. The responding cells are helper T cells which proliferate after recognition of allodeterminants on the stimulator cell. The proliferative response is determined primarily by differences at the B locus of the rat major histocompatibility complex RT.1 (130-132). However, proliferation can also be measured to differences at other loci within the RT.1 complex and to minor histocompatibility differences (133,134).

As was demonstrated by the transplant studies, T lymphocytes from BB rats failed to proliferate adequately in MLCs to either allogeneic Lewis or WF stimulating cells. Because lymphocytes from WF animals were able to respond to irradiated BB cells in MLCs, these results indicate, as did the skin graft results, that significant histocompatibility differences exist between BB and WF rats. Yet, BB lymphocytes were totally unable to respond both in vivo and in vitro to such differences. As was stated previously for the allograft and mitogen findings, the severe T lymphopenia especially of the helper T cells, probably contributed to the defective ability of lymphocytes from BB rats to proliferate in the presence of allogeneic cells. Most likely, the numbers of BB monocytes and accessory cells were sufficient for adequate proliferative responses by BB T cells.

T lymphocytes from BB rats obtained after passage through rabbit Ig-antirat Ig columns had enhanced but still severely decreased mitogenic and MLC responses in comparison with WF cells. Because the passage of spleen cells through these columns is thought to greatly augment lymphocyte responsiveness by removing suppressor cells (121), these results suggest that suppressor cells may in part contribute to the T lymphocyte unresponsiveness seen in BB rats.

The data concerning the effects of the addition of either irradiated or nonirradiated BB cells on mitogenic and MLC responses by WF lymphocytes were equivocal. In several experiments, some inhibition of proliferation by WF lymphocytes was noted in the presence of BB spleen cells, yet in other studies no suppressor activity by BB cells was observed. Imbalances between helper and suppressor T cells may indeed play a role in the T lymphocyte immunoincompetence in the BB rat. However, because the suppression of WF responses by added BB cells was modest and inconsistent and because BB spleen cells were good stimulators in MLCs, other factors besides increased suppressor activity must contribute to the lack of significant T cell-mediated immune responses by BB rats.

One aspect of the immune system of the BB rat that was considered was the ability of BB lymphocytes to respond and to produce IL 2. IL 2 is a lymphokine produced by helper T cells which has a broad spectrum of biological activities. One of the most important actions is that IL 2 appears to

serve as a second signal which is necessary for the continuous proliferation of activated T cells (116,135,136). The latter has been supported by evidence that T lymphocytes require IL 2 for proliferation after activation to mitogens and to alloantigens (117,120,137). Thus, the inability of T lymphocytes to respond to or to produce IL 2 could result in serious defects in normal T cell function. The Mrl/Mp-lpr/lpr mouse which develops both autoimmune and lymphoproliferative diseases has absent IL 2 activity (138).

With a few exceptions, irradiated WF cells, which were still capable of secreting helper factors such as IL 2, did not enhance mitogenic or MLC responses of BB lymphocytes. BB cells also did not proliferate as well as lymphocytes from WF rats to known IL 2-containing Con A sups, nor did the addition of these conditioned media augment proliferative responses of BB lymphocytes to Con A or to allogeneic cells in MLCs. However, after rabbit Ig-antirat Ig treatment, T cells from BB animals did respond better to Con A sup, but still to a much less degree than lymphocytes from WF rats. This positive effect is probably explained by the removal of suppressor cells.

Lymphocytes from BB rats were able to produce IL 2, although in many cases, not as well as WF animals. This finding suggests that sufficient amounts of IL 2 were normally present in BB mitogen and MLC reaction wells, and especially after the addition of irradiated WF cells or Con A sup. It is thus probable that BB lymphocytes are

deficient in their ability to respond to IL 2 or other helper factors. This defective T cell responsiveness to IL 2 could possibly be due to either the lack of or the presence of suboptimal numbers of receptors for IL 2 or other cytokines on BB cell membranes. The immunodeficiency observed in the BB rat may thus possibly be more the result of a primary defect in the T cell itself because of its inabilty to respond with the generation of IL 2 receptors to stimulation by mitogens or by allogeneic cells, rather than being a consequence of disordered immunoregulatory mechanisms. Obviously, these hypotheses remain to be explored.

The T cell defect most likely occurs after T lymphocyte maturation in the thymus, since responses by thymocytes from weanling BB rats to Con A and Con A sup were comparable to those by WF control thymocytes. Additionally, no abnormalities were seen histologically in BB thymuses in comparison with thymuses from WF animals. In contrast, spleens and lymph nodes from BB rats were observed to be severely depleted of T lymphocytes. These findings and the previous results suggest that T lymphocytes in BB rats have peripherally acquired defects in immunoresponsiveness. It is also possible that the T cell immunoincompetence is due to stem cell defects that appear late in T lymphocyte maturation which do not affect functions of immature T cells. Such a hypothesis is supported by a recent study demonstrating somewhat restored MLC responses to allogeneic

cells by BB lymphocytes after neonatal bone marrow reconstitution (139). However, these enhanced proliferative responses by T cells from BB rats were still less than half as great as responses by WF and Lewis cells, indicating that further factors contributing to the T lymphocyte immunodeficiency may be present in the peripheral circulation.

In contrast to defects in T lymphocyte function, gamma globulin levels were similar in both BB and WF animals. This result suggests that perhaps the functions as well as the numbers of B lymphocytes are unimpaired in the BB rat. Clearly, more specific indicators of B cell function such as IgG production and plaque-forming assays need to be performed before this supposition can be proven. A summary of the findings on the BB rat is shown in Table 22.

Most T lymphocyte immunodeficiencies seen in man and animals involve to some degree the B cell limb of the immune system. The BB rat appears to have immunological defects primarily of T cells. Both the nude mouse (140,141) and the nude rat (142-144) have similarities with the BB rat, such as deficient responses to mitogens, to allogeneic cells in MLCs, and to IL 2. However, the obvious difference with these athymic animals is the presence of what seems to be a relatively normal, functioning thymus in the BB rat.

Increased incidences of lymphomas and lymphoproliferative disorders are found in many immunodeficiency and autoimmunity syndromes (145). Such a relationship is well illustrated in New Zealand black/white F1 hybrid mice which

TABLE 22

SUMMARY OF FINDINGS

Findings	BB Rats	WF Rats
IDD	++	-
Susceptibility to infections	++	-
Autoantibodies		
PCA	++	-
SMA	++	+
Thyroid colloid antibodies	+	-
Thymocytotoxic antibodies	++	+
Immunological Parameters		
PMNs	↑	N[a]
Monocytes	N	N
Eosinophils	N or ↑	N
B lymphocytes	N	N
T lymphocytes	↓↓	N
Helper T cells	↓↓	N
Cytotoxic/suppressor T cells	↓	N
Immunoglobulin levels	N	N
Immunological Functions		
Peripheral lymphocytes		
Allograft rejection	↓↓	N
Mitogenic responses	↓↓	N
MLC responses	↓↓	N
IL 2 production	N	N
IL 2 responsiveness	↓↓	N
Thymocytes		
Mitogenic responses	N	N
Lymphoid Organ Histology		
Thymus	N	N
Spleen		
T cell areas	↓↓	N
B cell areas	N	N
Lymph nodes		
T cell areas	↓↓	N
B cell areas	N	N

[a] normal

develop diverse nonorgan-specific autoantibodies, defects in cell-mediated immunity, and malignant lymphomas (145-147). The BB rat appeared to have an increased frequency of lymphomas, all of which seem to be composed of B lymphocytes (data not shown in results). Such a finding might be expected, since most lymphomas are of B cell origin and because the numbers of T cells were so severely reduced in these animals.

Unlike other models for immunodeficiency associated with autoimmunity such as New Zealand black/white F1 mice (147), primary human hypogammaglobulinemia (148) and ataxia-telangiectasia syndromes (148,149), the BB rat appears to be unique in exhibiting T lymphocyte immunoincompetence in association with IDD. The disorder that appears to be most comparable to the BB rat is Type I autoimmune polyglandular syndrome seen in humans (1). The affected patients develop autoimmunity to most endocrine glands, albeit except the pancreatic islets, early in life. In addition, these patients have functional defects in peripheral T lymphocytes manifested by their development of overwhelming infections with Candida albicans and chronic mucocutaneous moniliasis.

Clearly, such severe defects in immunocompetence as observed in BB rats, are not found in human patients with IDD. However, more subtle immunological abnormalities may be present in human diabetic patients that have not yet been diagnosed. The T cell immunodeficiency may also be

unrelated to IDD in the BB rat. It may instead be a second serious disorder that occurs independently of autoimmunity in these animals.

Since lymphopenia and defects in peripheral T cell functions were present in all BB rats studied, regardless of age or the presence of IDD, it is difficult to visualize how a solely T cell-mediated autoimmune process could cause pancreatic beta cell destruction, resulting in IDD. The participation of humoral immunity, antibody-dependent cell-mediated cytotoxicity, and natural killer cell activity may instead by required for the development of IDD. Interestingly, Mycoplasma neurolytica and Mycoplasma pulmonis have recently been demonstrated to be potent B cell mitogens in the rat (114,150). Because BB rats have chronic infections of Mycoplasma mainly involving the respiratory tract, these results suggest the possibility that polyclonal B cell activation may occur in these animals. This activation of B cells could result in the production of autoantibodies to pancreatic islet cells, which either alone or with the participation of K cells, could be cytotoxic to beta cells, leading to IDD. Thus, the inability to transfer IDD with lymphocytes from diabetic BB rats could be explained as being due to the immunoincompetency of the transferred T cells or their minimal role in the pathogenesis of IDD in these animals.

In summary, the BB rat is severely immunocompromised with major defects in the T cell compartment. This

immunodeficiency may be a culmination of circulating T lymphopenia resulting from the presence of thymocyotoxic autoantibodies and increased suppressor activity. However, mature T cells from BB rats may also be deficient in their ability to function normally, including being able to respond to helper factors such as IL 2. The relationship between these separate findings of an autoimmune diathesis and profound immunoincompetence in the BB rat remains to be elucidated.

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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